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ANNUAL REVIEW OF PHYSIOLOGY

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PREFACE

The third volume of the *Annual Review of Physiology*, now presented to its readers, suffers from several deficiencies that are almost unavoidable. The most regrettable of these is directly attributable to the war and is reflected in the inability of many of our colleagues abroad to enter into full collaboration in authorship. Many of the Universities of continental Europe have practically suspended operations. In others, where work is still proceeding, there have been insuperable difficulties in procuring the scientific journals of Great Britain and America; in some cases there has been a complete cessation in the acquisition of foreign periodicals since the outbreak of the war. Under such circumstances it is clear that the *Reviews* must lose something of their international flavor.

It will be a disappointment to many, as it was to us, that the reviews expected from Professors Heymans, Bremer, and Bard could not be prepared. We are grateful to our colleague, Dr. Victor Hall, who generously aided in the emergency that thus arose by preparing, with but little notice, the review on the Peripheral Circulation. Knowing something of the extra obligations that have been imposed upon so many by virtue of the war we cannot express with adequacy the gratitude that we feel to all who collaborated in the authorship of the present volume.

Of necessity, the reviews differ in quality and approach. In several cases the reader may conclude that the author has striven to compile a comprehensive résumé of the year's work instead of critically analyzing selected papers of outstanding importance. Inevitably there will be lacking from such reviews an adequate appraisal of the field and an outline of the problems of commanding interest. Where this has happened we feel that it is the result of an honest difference of opinion on a matter where complete unanimity can hardly be attained. While we are fully cognizant of the service that is rendered by synoptic surveys that are terse in treatment yet comprehensive in scope, it is our considered opinion that this *Review* will find its greater value in presenting to the reader critical analyses and searching appraisals of the outstanding contributions of the year in selected fields. Such reviews will suffer, through the exigencies of space, from failing to mention

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many solid though modest investigations and may tend, without the exercise of scrupulous care and much wisdom, toward narrowness in perspective and prejudice in approach. We can only ask for a continuance of forbearance on the part of the reader and the further collaboration in authorship of those of our colleagues who, through an intimate knowledge of the subjects under review and an understanding of the difficulties in presentation of the subject matter, are peculiarly qualified to serve.

It may also appear, on cursory inspection, that not all of the subjects appearing in the present volume would lend themselves to annual or biennial review. Indeed, we have hitherto endeavoured to forego the selection of any topics that were not suitable for frequent and periodic survey. There are manifold difficulties in rigorous adherence to this policy and a word of explanation may be needed to clarify the problem as it applies to the present volume. The three topics reviewed by E. Bárány, R. Granit, and Y. Zotterman have hitherto been combined in a single review entitled "The Special Senses." The material which in previous volumes has appeared under "The Endocrine Glands" is now divided into two reviews, "Metabolic Functions of the Endocrine Glands" and "Endocrine Aspects of Reproduction." This division is necessitated by the expanding boundaries of the field and by the enormous numbers of papers calling for review. The two reviews by E. K. Marshall and W. Feldberg, each sharply restricted in scope, are parts of what we like to regard as a recurring section on pharmacology in which we would hope to bring to our readers those developments in the subject that are likely to be of greatest interest to the physiologist. The concluding chapter is a modest venture in applied physiology under which heading, in future years, we hope to give appropriate attention to industrial physiology, aviation physiology, and so forth.

Again we would thank our readers, especially those abroad, for their kindness in sending reprints of their recent publications. To others we are indebted for many friendly suggestions in respect to authors, topics, and editorial policy. To our editorial assistants we wish to express our thanks for much care and interest in the exercise of their responsibilities, and to the George Banta Publishing Company for the most cordial cooperation throughout.

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ERRATA

Volume II, page 75, line 27: *for* (198), *read* (211).
page 75, line 30: *for* (211), *read* (198).

THE RELATION OF BIOELECTRIC POTENTIALS TO CELL FUNCTIONING

BY GEO. H. BISHOP

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The majority of physiologists has been preoccupied with human or vertebrate function, and the electrical properties of tissues have been examined in greatest detail to explain the functioning of nerve and muscle in the animal body. Information on the source of the biological potential itself is less abundant and less familiar than is that on the behavior of potentials in such processes as excitation and conduction. It would be charitable to consider the many shortcomings of the following section of this review as a further example of the above circumstance.

Choice of the term "functioning" rather than "function" in the title is deliberate. While a function in conduction may be assigned to the potentials of nerve and muscle, the status of the more general question in plant and animal cells is illustrated by the answer received to the question, "What does *Nitella* need of an action current?" to which one physiologist opined, "Perhaps to stimulate its liver." As good an answer as can be given at present, the proposal has interesting implications, but they have not been followed up.

CHARACTER OF THE POTENTIAL

That the ultimate source of energy for potential development is metabolic is obvious from the failure of potential in asphyxia and other simular states. Concerning the more immediate source, several theories have been proposed, or better, theories exist concerning several factors which might account for potentials. All involve a cell membrane or interface upon whose "vital" character, as different from an inert artificial membrane such as cellophane, any disagreement with simple theory may be conveniently placed. One line of argument, going back to Bernstein, infers a membrane selectively permeable to organic or other ions, typically potassium, to account for "resting" potentials, while a temporary increase of permeability with excitation results in a current between excited

and other regions. Selective solubility of ions in phases whose interface acts as the membrane, may be considered as a variant of this, as may the inference of a charged membrane or one with critical pore size, or other factors which influence diffusion of ions (1). Another line of work emphasizes the colloidal state of protoplasm and its interfaces, and the effect on its physicochemical properties (such as gelation or cytolysis) under the influence of stimulating agents (63). A third assigns to the cell membrane itself an oxidation-reduction potential, with the assumption of electron conduction across it to enable it to act as an electrode (74, 78). The hypotheses developed are not obviously mutually exclusive, considering the complex behavior of the potentials. Chemical mediators have not so far been assigned an immediate role in potential production, although the complex interactions between potassium, acetylcholine, and potential may yet inspire such an inference. (See below.)

The potential as an oxidation phenomenon.—The view that cell potentials are of the oxidation electrode type is consistent with the widely accepted view that oxidation takes place catalytically at surfaces, but is more specifically argued from other considerations (74, 78, 79). For instance, Marsh found that currents applied to onion root tips altered their previous potentials in a manner characteristic of a storage battery rather than of membrane polarization (76) and that the temperature coefficient of the *Valonia* potential could best be interpreted as that of an oxidation process at a phase boundary in accordance with Lund's (74) theory of flux equilibrium (79), recognizing the fact that salts would affect such a potential as an element in the environment. The spectral distribution of the light effect on *Valonia* potential, corresponding to the absorption of chlorophyll, is assigned to the oxidative sequelae of that absorption, without any direct effect on membrane permeability (81). The temperature coefficient of the resting metabolism of nerve corresponds to an oxidation (hydrogen transfer) process, which is considered by Shapiro (107) to be at least the limiting reaction. It may be noted that these results apply to resting potentials of a steady state, and the end result may be determined by a reaction giving rise only indirectly to those products (ions) active at a cell interface where a potential difference is maintained. However released, at any rate, diffusion of ions to other interfaces than the locus of origin should set up potentials secondarily (93).

Experiments on the potentials of frog skin, obviously a complex structure, illustrate this. Amberson (1) refers to a diffusion superposed upon some other factor affecting the membrane and cites critical changes at the isoelectric point of some protoplasmic constituent. Dean (42) discusses the potentials in terms of ionic mobilities and the interaction of both anions and cations, judging the locus of action by the diffusion time required for a given effect. Barnes (14) finds the temperature coefficients of different skins are grouped about three values, characteristic respectively of oxidation, of some chemical process such as hydrolysis, and of simple diffusion, and infers that one or the other is the limiting one in different conditions of the tissue. Deriving its energy from oxidation, the measured potentials are attributed to diffusion of the resultant ions. Nerve stimulation may change the potential in either direction depending on its initial magnitude, while acetylcholine increases it if applied to the outside, decreases it inside, without altering oxygen uptake (12, 84). With equilibrium conditions there is a linear relation between resting potential and the change with nerve stimulation as if even this complex tissue, under constant environment, contained a potential element of which the action current were a simple negative variation of the current of rest (83). The frog skin in which oxidation is depressed by iodoacetate can maintain its potential on certain carbohydrate degradation products, this fact indicating a carbohydrate oxidative metabolism of the type found in muscle (52).

The potential as a function of colloidal structure.—The most critical statement that can be made about the surface which is the seat of the biological potential is that it is living, for no artificial membrane has been found to duplicate it adequately. The phase boundary between water and oily media show some of its properties (105). Hober *et al.* (65) picture it as a phase boundary affected by anions, cations, and organic substances dissolving in it, the change in the labile surface with activity being a partial and reversible cytolysis. Excitation might involve a change toward dispersal of colloids, due to the transitory production of cytolytic agents by the stimulus. Heilbrunn (63) reports precipitation of the muscle colloids and shortening, as calcium chloride penetrates from a cut end, and relates the effects of calcium and magnesium to a colloidal chemical theory of stimulation (62) according to which excitation involves mildly cytolytic changes in colloidal state. The action of

alkaline earths on the potential of crab nerve, as a stabilization against the changes involved in excitation, is discussed as a change in permeability of a membrane and as a change in the partition coefficients between it and the adjacent protoplasm (60). Similar ideas appear in the references on diffusion potentials.

The potential as a diffusion process.—Osterhout and others (86 to 93) in a long series of papers have studied the relation between potential and permeability of the plant cell for salt ions. The more recent of these (64, 88 to 92) deal with apparent ionic mobilities and partition coefficients. The conclusions are drawn that potentials are assignable to differences of ion concentration, particularly of potassium ion, across cell surfaces, with the further circumstance that the permeability of the surfaces may be changed by various agents. Differences of ionic concentration are assigned to forces set up by the cell's metabolism acting particularly through acid metabolites, notably carbon dioxide. Action currents result from a local and reversible change in membrane permeability. Anions are involved in controlling the state of the membrane (21) and the outer surface is extremely labile (20). Complicated behavior of such potentials is explained as the sum of the actions at each layer, since at least two interfaces and a layer of protoplasm intervene between cell vacuole and cell surface, and the effective cell membranes appear not to be homogeneous structures (93). Ions released at one locus may cause complex fluctuations of potential as they diffuse to other cell interfaces, so that any agent affecting the state or reaction of protoplasm or its interfaces might thereby affect the cell potential.

Physical properties of the electrical circuit.—Measurements of resistance and capacity of the giant axon of the squid, in both the unexcited and excited states, definitely confirm the theory that a change in permeability accompanies excitation in nerve, as has been demonstrated for plant cells, and add further evidence that the process of excitation is a general one (36, 37). More recently Curtis & Cole (41) have measured the impedance across a single membrane from inside to outside the axon during activity. According to the report by Cole (34) supplemented by a further one in press, an electrical mass or inertia factor has been detected which appears in measurements as inductance, and which, with the other factors of impedance, offers a basis for interpreting the oscillations of potential and of excitability which have been observed by others.

The analysis of nerve excitation which is developing involves linear functions of impedance and of potential source below threshold, with a difference of resistance in opposite directions making the membrane a good rectifier; it further involves changes at the threshold of excitation in the resistance-rectifier mechanism and in the potential source, i.e. these functions become non-linear, with no significant change in reactance. Whether the change in resistance is alone sufficient to account for the output of energy from the potential source, or whether resistance-rectifier and potential factors are functions of a common element is perhaps not yet clear. Offner *et al.* (85) have proposed an equation for conduction involving a change only in resistance, the nerve "battery" remaining active during excitation.

Measurements of conduction rate of the squid axon show an increase with decrease of external resistance, this fact confirming the idea that in conduction the inactivated region is stimulated by current flow from the site of activity (68). The change of external resistance, while it changes recorded amplitude, induces no change in duration (75). Artifacts produced in the nerve record by changes in external resistance depend on the spatial distribution of the resistance change. Electric circuits that integrate or differentiate the action potential curve offer another method of evaluating the properties of excitable tissues (103). Linearly increasing currents change the factor for accommodation in nerve as the threshold rises, increasing λ at the anode and decreasing it at the cathode and suggesting a change in the potential source without excitation under these conditions (106). But Parrack finds no accommodation in frog nerve with circulation intact, the phenomenon developing under anesthesia or stoppage of blood flow (94, 95). Modification of two-factor theories of conduction is suggested. The theories of Rashevsky and of Rushton are shown to be essentially equivalent (111).

Dipoles v. the membrane hypothesis.—While it has been shown (119) that the principles for analysis of potential fields introduced by Craib may be reduced to common terms with the conventional membrane hypothesis, the choice of heading for this section is intended to epitomize the peculiar animus which has seemed to characterize from the start the conduct of potential analyses in accordance with the Craibesian postulates. This remark is in no way intended to disparage the results obtained, nor the principles

employed in their interpretation; current publications register considerable abatement of the attitude.

In fact, ignoring the nature and disposition of the source of potential within the tissue, the physical methods of treatment embodied in this approach offer some advantages in dealing with potential fields as ordinarily recorded in the surrounding medium. One of these advantages is that the situation can be dealt with precisely even though the source of potential is ignored. Two current papers deal with the doublet theory of potentials, one (16) giving a general solution of equations for the field surrounding heart muscle in terms of the electric moments of dipoles, the other (10) interpreting in terms of the dipole analysis records believed to have been previously misinterpreted (55) because of a misconception of the membrane hypothesis. Whether a more acceptable version of the membrane hypothesis would have been more effective is not apparent. An ingenious method of leading monophasic potentials from the heart, with one electrode applied to a region blocked by cold, enables records to be taken which can be interpreted in accordance with the dipole schema (11). Records of electrical and mechanical events in the heart indicate that contraction is coincident with the maximum current flow, this finding offering evidence that the normal stimulus is electrical (56). A study of the distribution of charges in the active gastrocnemius muscle of the frog (57) in terms of "unipolar" and "differential" potential curves might be correlated with the monophasic and differential curves derived by electrical methods (103) and interpretable presumably in accordance with the membrane theory.

Interaction of the electrical process with the chemical environment.—Without going into the question of chemical mediation of nerve impulses, certain complex relationships between ions and possible mediators may be briefly noted. The relation of these data to potential is not clear at present.

Potassium is reported to liberate acetylcholine from muscle (25) and from sympathetic ganglia (26). Low calcium blocks synaptic transmission because it prevents the release of acetylcholine, while high potassium does not, the absolute and not the relative value of calcium being critical (61). Increased calcium raises the threshold of muscle to acetylcholine (17). Cobra venom, like curare, prevents the response of muscle to acetylcholine, although potassium still causes a response (32). These findings lead to the

conclusion, contrary to the views of Brown *et al.* (25, 26), that acetylcholine is the precursor of potassium at the neuromuscular junction. To test this (33), Cicardo & Moglia perfused frog muscles with Ringer's minus potassium plus acetylcholine, whereupon potassium was liberated. A tenfold increase occurred after nerve degeneration. After curare block, no potassium appeared in the perfusate.

It might be worth while to consider such results from the point of view of the work on plant cells reviewed above, as well as from the viewpoint of synaptic transmission. Considering that these and many other agents employed to modify the action of excitable tissues do affect the potentials observed, there is room for further consideration of the potential mechanism as acting in a chemical environment. At present it is impossible to draw a distinction between the environment in which it acts and the potential mechanism which is acting, and perhaps such a distinction does not exist; discussions of the effects of various agents on potential seem to draw this line as dictated by convenience rather than by logic.

The state of our information concerning biological potential sources is reflected in the preoccupation of much of the literature with the elaboration of theoretical explanations, a feature which usually characterizes an equivocal and complex field. The work involving physical measurements is least affected with this attribute, and offers prospects that are correspondingly hopeful. This tendency may be expected to take care of itself moreover, for a theory is rendered metaphysically innocuous either by its disproof as theory or by its substantiation as fact, and meantime serves as a potent stimulus to experiment. Still, a report in which the data have been so lavishly ornamented by the citation of hypotheses justifies, perhaps demands, of the writer a surmise of his own: that any theory of bioelectric potential so far devised should be taken with a liberal dose of its own saline constituents.

THE COURSE OF EXCITATION IN NERVE AND MUSCLE

Certain conductile tissues in animals offer an advantage over plant cells for the observation of potentials, in that only one active layer is involved, instead of at least two. Recent refinements of manipulative technique have made possible the recording of potential changes in narrowly circumscribed regions of single units. In certain cases a complicated series of potential events can be demon-

strated as concomitants of excitation, consisting of at least three steps: physical polarization of the tissue by the stimulus (or by the impulse in conduction), a local physiological response below threshold for conduction, and the propagated action current.

The local process in nerve and muscle.—The local process arising under the cathode in invertebrate nerve, superposed upon the physical polarization effect of the electric stimulus, appears to have been considered from the first as essentially the conducted response process, too low in amplitude or occupying too small an area of the nerve membrane to reexcite adjacent regions (66, 70). While no very specific conclusions appear in this regard, a question may be again raised by recent work. In the squid giant axon at locally depressed regions, the line between electrotonic and propagative phenomena becomes vague or nonexistent, and there occurs incremental or decremental conduction of what might be either a local response or a propagated one (102). The two components of the local effect of a stimulus, polarization and local process, add in terms of excitation, as they do in terms of potential, the local response showing propagation near threshold, followed by a refractory period similar to a conducted response (97). However a local process sums with a propagated response above threshold at the site of stimulation (104). When plotted against stimulus strength, the amplitude of this process rises along an S-shaped curve, from 50 per cent to well over the limen. The greatest fluctuation in latency in the crab axon appears at the middle steeply rising region of this curve. This region is also the threshold, where the amplitude of the local process is changing most rapidly with stimulus strength. This correlation of latency in excitation with local process is compared with the original observation on latency of Blair & Erlanger, although the latter could record no such local response in frog nerve as is observed in the invertebrates. The critical question would inquire what is happening to the nerve membrane during the various manifestations of its activity. The work of Cole suggests a (momentarily) irreversible change in the resistance and E. M. F. as a critical factor in excitation, and further measurements of impedance during the development of excitation may relate the different events to a common term. At any rate evidence is increasing as to the close relationship between the state of excitability and the potential level, however the latter is modified.

Consistently with the above, the latency of response for a given stimulating value of current is longer after long-lasting electric stimuli than after brief ones, but latency for the latter lengthens near the threshold (58). Under galvanic current polarization, the time course of the excitation following a shock is not altered, although the quantity of current required to stimulate varies with the degree and direction of polarization (47). On the other hand, with rectangular current stimuli, the degree of polarization attained at threshold excitation is not constant, it being a minimum at the rheobase and increasing with decreasing duration of the stimulus (44). Catelectrotonus diminishes the latency and anelectrotonus increases it up to a value of 70 msec. in frog nerve (45). In view of Parrack's observations on the occurrence of negative accommodation (95), one might suspect the involvement of some such factor in these long latencies. See also the effect of polarization on accommodation, discussed above.

The data on the local process at the motor endplate in muscle reflect the greater complexity of structure here as compared to a point locus on uniform nerve. Its importance as a junction transmitting impulses is critical in proportion. The local response is obtained clearly only after modification of the tissue, either by repetitive stimulation or by drugs such as curare (28, 43, 100, 101). Two potential signs of activity occur, spike and local response. The latter has a slow decay following a sharp rise, and is interpreted either as polarization of the endplate region by the nerve action current (101), or as a persisting excitatory state, separate from the "detonator" activity represented by the conventional muscle spike (43). These two notions are not inconsistent, but there is disagreement as to causal sequence, one view being that the local response is the direct precursor of the spike, quite as in the local response of nerve (28, 101), the other that they are parallel responses, presumably assignable to separate mechanisms (43). The evidence for the latter is that the spike may start before the beginning of the nonconducted response. Evidence for the causal sequence is that, as partial block is removed (by decreasing rate of nerve stimulation, for instance) the spike occurs earlier, finally taking off from the rising phase of the local response but not observed to precede it (101). The most extreme position (28) on this critical point is that functionally two junctions exist, nerve to sole and sole to muscle, with the local response set up in the inter-

mediate sole, the latter recognized as a specialized region of the muscle fiber substance capable of being excited by the nerve action current, rather than as a morphologically separate structure. Further implications of functional segmentation in muscle are seen in a longitudinal potential difference, proportional in magnitude to the number of muscle segments subtended by the two electrodes (27).

In a series of papers on single muscle fibers, Wilska (112 to 116) has reported tests of the effect of pricking and of electric stimuli. He finds that pricking with a fine electrode results in a tetanus, that no potential appears without a contraction, and in fresh preparations no contraction occurs without a conducted potential, this observation being contrary to the report of Gelfan & Bishop (54). Differences in technique are suggested as the occasion for the differences in observations. An interesting finding is that conduction rate increases with stretch, a 50 per cent increase in length being associated with a 10 per cent increase in speed. Temperature coefficients of conduction rate are not constant over the range of 0 to 36°C., the change not being linear below 10°C. Feng *et al.* (48, 49) have observed electrical negativity in whole muscles after block of muscle contraction, and repetitive discharges to single stimuli after a tetanus, with or without physostigmin, a more detailed analysis of which findings might be made on the basis of the observations on single fibers.

Other properties are associated with the local response of muscle over the nerve endplate. No absolutely refractory period accompanies it (108, 109), and a "local" response of the whole muscle is elicited by electric stimulation after treatment with excess potassium chloride, but this is presumably the idiopathic contraction observed by earlier authors under the cathode of a galvanic current and below threshold in single fibers (54). No nerve impulse activates muscle without setting up the characteristic local response, which may also be induced by direct stimulation of the region, while a spike may or may not follow (29). Direct stimulation of deeply curarized muscle gives a local response when no other excitation of the muscle, at the endplate or elsewhere, is possible. (The present writer's interpretation of a complex discussion is that the endplate region requires a higher concentration of curare to block it than does the rest of the muscle.) After application of acetylcholine which blocks a nerve impulse, a direct stimulus

to the endplate results in a response at the endplate; a first application of acetylcholine produces a tetanus, after which the region is refractory to a similar application. Also the nerve-sole junction can be blocked by acetylcholine without blocking off from the rest of the fiber an impulse started at the endplate (28). The local response to a nerve stimulus is decreased by physostigmin; it is blocked by curare only after blocking of the conducted response; further, under curare, the spike falls out as the local response decreases in amplitude (101), quite as at the stimulated point of invertebrate nerve. The local process shows summation, facilitation and latent addition, calcium increasing these related functions, acetylcholine decreasing them to the obliteration of any response in physostigminized muscle. Since curare blocks the local response to a nerve impulse without preventing the release of acetylcholine, while physostigmin reduces the response but prevents the destruction of acetylcholine, it is concluded (101) that acetylcholine cannot be the mediator between nerve and endplate, but may act as a regulator of electrical transmission of the nature of depolarization. Critics of the electrical theory of transmission may perhaps be found to whom this argument is not ultimately conclusive.

The nerve as synapse, and vice versa.—Gasser (53) remarks circumspectly that "the events which take place in those parts of the neurone entering into the synapse may resemble, qualitatively at least, events taking place in other parts of the neurone." The writer of the present survey also confesses a bias toward electrical conduction across the synapse, to the extent of feeling it profitable to review the electrical behavior of altered regions of the nerve axon as a significant model of synaptic function.

To construct an artificial synapse one proceeds, with tongue in cheek, to produce a narrow region of depressed irritability in a nerve fiber, whereupon, barring too extensive damage at the critical locus, an impulse fired into one side of the depression emerges, after some travail, beyond it. Other modifications of excitability lead to phenomena sufficiently resembling the functions of a proper synapse to permit any competent physiologist to fill in the details. However the outlines of the picture are undoubtedly there, conjecture leads to experiment, and the theory of synaptic transmission hangs on the issue. The performances of some of these models are dramatic.

The impulse in medullated nerve may jump a polarized depression by unit internodal segments (19), while a potential observed below a block (67) is assignable to a polarization effect of the action current flowing across it, accompanied by the appropriate changes of excitability. Lorente de Nó blocked impulses with cocaine and studied in great detail the currents flowing through the nerve below the block (73); from considerations both physical and histological he discussed the application of such results to the synaptic junction. The fact that an impulse in one axon, after but reasonable departure from normal conditions, may be induced to stimulate a similar axon adjacent to it (69), the arrangement of the two involving a tolerable approximation to that at synapses, adds a further air of verisimilitude to such a discussion. From this to the work of Barron (15) is an easy step. Failing to confirm the existence of the recurrent fibers in dorsal roots originally reported by Barron & Matthews, he infers that the discharges from one dorsal root when a different one is stimulated are mediated across such a virtual synapse between two adjacent fibers embedded in the spinal cord. The short synapse time obtained precludes their being of the type of dorsal root reflexes reported by Toennies; further, the finding (69) that in excised parallel single axons the effect of activity in one upon the excitability of the other is very materially a function of the shunting resistance, must give evidence of surprising irritability in the axons involved in the cord conduction. If nerve fibers can so conduct, the transmission across real synapses by nervelike processes is rendered plausible.

The effect of one such fiber on another is found to be a polarization by the action current, with corresponding changes in excitability of the affected fiber developing (71). As the spike approaches the common electrode of record, the inactive fiber becomes first positive and less excitable, then negative and more excitable, these phases being followed by a third phase like the first. If both fibers are activated simultaneously the conduction rate of each is reduced; if asynchronously, the leading impulse is slowed, the following impulse accelerated, these effects tending toward synchronization of the impulses.¹

¹ A similar procedure has been carried out by Blair & Erlanger (paper in press) in multifibered nerves, one branch of which is stimulated, while records are taken from the other after a submaximal stimulus to the common trunk. They find a

A state of excitability appropriate to the requirements of "synaptic" passage from one fiber to another is readily induced in intact single fibers of invertebrates, either by low calcium concentration or by excess sodium chloride. An "experimental synapse" consisting of two overlapping fibers with sodium chloride at the point of contact permits an impulse in one to set up an impulse in the second; this preparation is one peculiarly accessible to investigation (7). Under conditions of enhanced irritability a fiber may become spontaneously and repetitively active, while below this stage, the passage of the impulse is facilitated by rhythmic fluctuations of potential (local process, prepotential) and of excitability, in which two frequencies may be observed (8). One has a period of about 8 msec., and these oscillations are modulated in a period many times as long. The minimal synapse time, less than 3 msec., appears when the impulse in the sending fiber arrives at the junction at the crest of a negative spontaneous wave in the receiving element (7). If, however, the impulse arrives during the low amplitude phase of the modulation (8), the impulse may not pass immediately, but serves to increase the energy of the system. The fiber then responds much later, and "spontaneously," at the phase of highest amplitude, when without the added impulse energy the threshold would not have been attained. Some preparations respond twice, once at the time of the first negative wave following arrival of the impulse, and again at the time of maximum amplitude of an incrementing train of oscillations. Finally, if spontaneous activity occurs, its frequency is not that of individual subthreshold waves but rather of the maximal or the threshold unit of each incrementing series.

The controlled development of these phenomena may be observed in normal fibers under the influence of polarizing currents (2) and, of course, with low calcium concentration. With increasing excitability of the preparation, a negative local response first develops a positive after-effect, then a second negative, etc., to a decrementing series, which may, near rheobase, become an incremental one (4). The duration of the local response is greatest with

depression of apparent excitability during passage of the impulse in half the fibers of the common trunk, followed by a rise, which is assigned to a lowering of resistance in axons activated in one half of the trunk, shunting the other half. When a region near the point stimulated is crushed, these changes are reversed, presumably due to polarisation of the nerve by its own demarkation current.

stimuli (constant currents) which last through it (3); low calcium concentration lowers spike height and rheobase (4); further, current polarization affects complexly the period of oscillation and its decrement (5). At anodal opening, the oscillation of potential also follows (6), stimulation occurring in the negative phase, which suggests amendments to Pflüger's laws (9). According to this account the postcathodal depression is not a direct result of current flow (6), but a secondary effect of excitation to the extent of local response, and is represented physically by the positive phase of a typically oscillatory phenomenon.

Oscillatory phenomena have been previously studied in terms of excitability levels in frog nerve stimulated by alternating current (82). The finding of an optimum frequency for such stimulation led to the concept of resonance, which has been studied also on models and formulated into equations. Coppeé finds that a coefficient of resonance, λ/K of Hill's equation, changes with temperature, as well as with the customary manipulations of calcium concentration (38). Below the value of 3.3 the nerve becomes spontaneously active. These results are related to the spontaneous activity in nerves from winter frogs. The period of free oscillation of the excitability deviates from the optimal frequency of stimulation, or resonance frequency, by reason of the effect of current flow on the preparation (39). See also the paper of Shanes (106) for discussion of the effect of current on accommodation. It will presumably be possible to relate these phenomena to changes in physical values associated with nerve function, such as the measurements of impedance at threshold (41), but at present it is not clear what essential distinctions are to be made between electrotonic polarization, whether by external currents or by intrinsic activity, local response to a stimulus of nerve or muscle, and spontaneous oscillations of potential in hyperexcitable states; we might add to this sequence the action current itself.

The fluctuation of latency in frog nerve, studied statistically, appears to follow a random distribution (96); accordingly a random distribution of ions responsible for excitation is presumed to lie back of it. This seems at variance with the results on invertebrate axons, where a virtual sine function of the oscillation of excitability should lead to a cosine distribution of latency even if the amplitude varied in a random manner. While it is not the ambition

of this writer to outdo the authors cited, it is obvious that the topic has not yet been exhausted.

One passes with a sense of let-down from teasing the ersatz synapse to the untortured contemplation of its natural prototype, the normal ganglion cell. When the pattern of stimulation on pre-ganglionic fibers is varied from the usual simple train of volleys to a distribution more statistically comparable to the presumed normal incidence (24), a single cell of the stellate ganglion no longer responds exactly once for each stimulus, but with a frequency determined by both the number and the frequency of the impulses impinging upon it; i.e., both spatial and temporal summation are effective at the synapses of a single cell.² It is probable that during stimulation each postganglionic impulse is immediately set off by some one preganglionic impulse, arriving upon a background of enhanced excitability, this being the so-called detonator action of the impulse. But during the after-discharge that follows adequate stimulation, the responses must be assigned to a persisting state of excitation above threshold. The observation of such an after-discharge from a single neuron may offer a basis for interpreting certain reflex responses in terms of circuits as simple as some that have recently been recorded (99), but after dealing with the modern axon the reader is puzzled whether to consider the synapse *au naturelle* as being more complex than necessary, or too simple to be plausible. One will be resigned however, to the finding that calcium concentration affects the result.

In relatively normal frog nerve, subjected only to dissection and anodal polarization (46), certain complex behavior, characteristic both of the synapse and of the neuromuscular junction, can be elicited without discontinuity of structure. A degree of block sufficient to stop one impulse may be overcome by a tetanus (facilitation), due presumably to supernormality following each, but after interruption of the tetanus, its renewal finds blocking more complete than initially (defacilitation), thus imitating at least the process of extinction. The effects are assigned to electrical influence

² In a recent paper, unfortunately overlooked before this review was in type, the foregoing procedure is elegantly duplicated on the crayfish ganglion, employing normal sense organ stimulation and direct recording of both afferent and efferent single-fiber discharges. The same conclusions apply, and effects of various agents on the synapse are recorded (96a).

below the block from the activity above it. If the processes at junctions should in fact be explainable on the same basis, the role of supposed chemical mediators would fall into an auxiliary category; while on the basis of the finding (30, 31, 72) of the latter substances in nerve trunks, it would seem to be incumbent upon advocates of the electrical theory of conduction to assign these substances a role in such events as those just described, for it is an axiom of the experimental method that no accidents occur in nature.

Cell potentials.—By cell potentials, one refers to a potential associated either with the neuron body or with its dendrites, as distinct from axon potentials, and the interest in such problematical entities is in direct proportion to the difficulty of demonstrating their existence. The difficulty arises from the fact that wherever one finds cell bodies one also finds such a profusion of axons that identification is precarious. Only by the use of microelectrodes so arranged as to record chiefly from a region comparable in dimension to a single cell, and then only by comparative records taken at different distances from known cell layers, is it safe to differentiate between responses. The importance of such investigations derives from the association in nervous tissue generally of activity with a characteristic potential form, and an interpretation of synaptic function in terms of such potentials would go far toward solving the problems involved in synaptic conduction.

The required procedure has now been successfully applied in the hippocampus (98) and in the cervical sympathetic ganglion (110). From the former are recorded three types of activity, slow spontaneous waves, apparently simple, 20 to 70 msec. in duration; rapid negative spikes similar to those of axons but largest near cell bodies; and diphasic responses to stimulation, 10 to 20 msec. in duration. Indications are at present that these are assignable to various modes of activation of the pyramid cells and their perikarya. In the sympathetic ganglion the responses are not greatly different from the known spikes of axons found there, but from their sharp localization and all-or-none character they are inferred to be responses of single cells, with after-potentials which are different in form from those conventionally recorded from sympathetic fibers. These results suggest similarity of response in axon and cell body in the outlying ganglion, but difference in form of discharge

associated with the more complexly integrated central pyramid cell.

Advantage has been taken of the rather simple structure of the insect retina (40) to record responses which may turn out to be comparable to those of single elements, if one can assume that single elements are being recorded in parallel. At low light intensity the responses are diphasic, becoming monophasic on stronger stimulation. The *a*-, *b*-, *c*-, and *d*-waves characteristic of the vertebrate retina finally develop in a retina consisting essentially of one layer of active elements. From this one might infer that even in the vertebrate eye the retinal potential recorded is chiefly that of the sensory elements. The results are comparable to those of Hartline on the eyes of invertebrates, provided that one can infer that the potentials are those of retinal elements rather than of nerve cells in the ganglion lying not far behind the retina. While the authors suggest the latter possibility, there is no reason to suppose that ganglion cells rather than sense cells would give potentials of the form observed.

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THE PHYSIOLOGICAL EFFECTS OF RADIANT ENERGY

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Skin.—The specific effects of long wave ultraviolet radiation are different from those of short wave. Following irradiation with energy extending from 320 to 480 $m\mu$ a marked, deep red erythema is produced. The maximum effect is observed at 385 $m\mu$, with two weaker maxima at 360 and 408 $m\mu$. If the intensities are so chosen that the same degree of erythema is produced at 297 and 385 $m\mu$, that at 385 $m\mu$ reaches its maximum in two to three hours, while that at 297 $m\mu$ is just becoming noticeable. Twelve hours after irradiation both show maximal reddening, but the color obtained at 297 $m\mu$ is carmine red, at 385 brown red; forty-eight hours after, the erythema produced at 385 $m\mu$ is brown, that at 297 $m\mu$ still maximally red. Five weeks later, that at 385 $m\mu$ is still strongly brown, while that obtained at 297 $m\mu$ is barely pigmented. A slight erythema at 385 $m\mu$ produces marked tanning, the same degree of erythema at 297 $m\mu$ hardly any. The erythema threshold at 385 $m\mu$ is 500 times greater than at 297 $m\mu$. The three maxima at 360, 385, and 408 $m\mu$ are probably related to absorption by specific molecules in cells deeper than the horny layer. There is similarity to the absorption maxima of hemin. Irradiation with energy with a maximum at 385 $m\mu$ has special significance in the treatment of tubercular conditions, at 297 $m\mu$ in the treatment of rickets (48).

There are several contributions to this relation between specific effects of shorter and longer ultraviolet radiation and the production of erythema and tan, including contrasts between the action of sunlight with its wealth of longer ultraviolet rays and its poverty in the shorter, and the energy emitted by various artificial sources (52, 71, 80, 87, 113).

The pigmentary changes in the skin have been objectively determined, spectrophotometrically, after irradiation by sunlight (19, 20). The curve of the first effect (hyperemia) indicates active arterial blood flow; later the curve shows a shift from oxyhemoglobin to reduced hemoglobin indicating blood stagnation and decreasing

local blood volume. An increase in melanin is indicated by a depression of the violet end of the spectrum. The degeneration of melanin produces an allied pigment, melanoid, shown by a flattening of the curve near 400 $m\mu$. The primary precursor of melanin is tyrosine and the formation of "dopa" may occur in the skin by the action of ultraviolet rays if nonspecific oxidation catalysts are present (111).

Sex hormones have significance in tanning, which may be a photographic-like process of exposure and development with the sex hormones acting to develop color-lacking material laid down in the skin by exposure (40, 41). Individual variations in the degree of pigmentary alterations during pregnancy may be also related to characteristic differences in the metabolism of these sterols (39). A film of sweat, due to dissolved and suspended substances, partly screens the skin against erythema-producing radiation (14). Many of the substances or preparations for which protective claims are made are valueless (5, 51, 62).

Is histamine, or some histamine-like substance (H-substance) produced or set free in the skin following ultraviolet irradiation and other forms of skin injury (72, 73)? The properties of the unstable enzyme which converts histidine into histamine in animal tissues have been described (135). Evidence, direct (123) and indirect (23, 24, 26), has been presented that ultraviolet irradiation increases the amount of H-substance in skin and blood. Ultraviolet rays exert an inhibitory influence on the cutaneous reaction due to intracutaneously injected histamine in nonallergic subjects, but increase the reaction in allergic subjects, due to their constitutionally lowered ability to elaborate histaminase (2). It is suggested that the nature of the tissue injury caused by radiant energy resembles the effect of coagulative necrosis by mercuric chloride and is different from that produced by venoms and staphylococcal toxin (60).

From an analysis of the erythema curve and the pharmacological action of ultraviolet radiation there seems little room for doubt that an active substance is liberated, which is responsible for the erythema response, as an indirect capillary reaction. Apart from the vasodilatation, which appears after a latent interval, the structural changes are limited almost entirely to the stratum mucosum. The erythema response is due to photochemical decomposition of some constituent of these cells with the liberation of active reaction

products, which then diffuse to the region of the minute vessels of the subpapillary venous plexus of the corium and lead to vasodilatation. The probable chemical nature of the photolyte is suggested by several independent lines of evidence, and consideration of its properties provides strong experimental support for its identification as a typical protein or a simple derivative. In ultraviolet erythema a physiologically active macromolecule is produced. From the amount of energy constituting the minimal erythema dose and the absolute quantum efficiency of the total photochemical changes in typical proteins and the concentration of protein decomposition products, the direct photochemical production of H-substance from the proteins of the stratum mucosum is reasonable from a quantitative point of view. The photo-oxidative decomposition of typical proteins with energy of wave lengths 235 to 315 $m\mu$ leads to the formation of proteoses and similar breakdown products. The hypothesis that the H-colloid is to be identified with proteoses formed photochemically offers a possible explanation of a number of the observed phenomena of the action of ultraviolet radiation (88).

Observations on a case of urticaria solare (1) lead to the conclusion that neither histamine nor a readily diffusible H-substance of low molecular weight is responsible for the skin response to ultraviolet irradiation. On the other hand it has been demonstrated that irradiation with a carbon arc definitely increases the content in skin and blood of an H-like substance (64). It is worthy of note that careful work (85, 86) negatives the view that there is an H-like substance in exudates or in their partially purified active fractions and renders untenable the H-hypothesis to account for the mechanism of increased capillary permeability in injury.

Work is being continued on the cell-proliferation-stimulating, growth-promoting substances produced by cells injured by ultraviolet radiation. These factors are effective in such small quantities as to permit the suggestion that their action is hormone-like (119). The production of these "wound hormones" has been confirmed by a more quantitative method, thus affording data as to the relative potencies and yields in various preparations (76). They are considered to be the natural stimuli to the repair processes in animal tissues following injury (78). They are somewhat nucleic acid-like (77).

Direct irradiation with a carbon arc of artificially produced

wounds in dogs indicates but little, if any, accelerating effect on the healing of a clean wound (122). On the other hand sterilization of wounds and of extensive areas of unhealthy granulation tissue can be successfully accomplished by ultraviolet radiation (31), most efficiently so by a special unit in which over 80 per cent of the output is at $253.7\text{ m}\mu$ (45). Healing is better, there is less danger of infection and less local and systemic reaction.

The effective emissivity of the skin in the infrared, of importance in reading skin temperature by the radiation method, in air conditioning, etc., is between 98 and 99 per cent (42). The penetration of red and infrared is noteworthy only between 0.7 and $1.3\text{ }\mu$ with a maximum between 0.7 and $0.8\text{ }\mu$ (51, 53).

Eye.—There have been claims that certain individuals could actually see ultraviolet energy (72). A recent report (32) describes the color sensations of the author's left eye, the crystalline lens of which had been destroyed. His retina is sensitive to wave lengths as short as $310\text{ m}\mu$, the sensation for 360 to $310\text{ m}\mu$ being blue. The sensitivity is not due to fluorescence of the retina. The owl responds to ultraviolet radiation of $360\text{ m}\mu$. Whether this is actual vision or due to fluorescence of the ocular media remains to be decided (49).

Lens protein, extracted in 0.5 per cent potassium chloride at pH 7.2 shows no increase in opalescence after exposure to ultraviolet radiation at 4°C . followed by heating to a moderate temperature. However, extracts in 0.5 per cent potassium chloride or Ringer solution, containing varying amounts of calcium chloride, develop an opalescence when irradiated and heated, which increases with the amount of calcium present (13). Thus it would seem that ultraviolet radiation (300 to $315\text{ m}\mu$) which penetrates the cornea but is absorbed by the lens is capable of denaturing lens protein so that opalescence develops provided small amounts of calcium salts are present in the lens. When parathyroid extract is added to the solution in which lenses are immersed the penetration of calcium into the lens is completely prevented. This suggests that low blood calcium after parathyroidectomy is due to loss of calcium to the cells and that cataracts which then occur are due to denatured lens protein provided that the eyes have been exposed to ultraviolet radiation, and to greater permeability of the lens capsule to calcium in the absence of parathyroid hormone.

Circulation.—The satisfactory results obtained in prophylaxis

of postoperative thrombosis by ultraviolet irradiation of the operation field have been explained as being due to the opening up of arterial-venous anastomoses (66). The increased amount of blood in the liver and skin resulting from ultraviolet irradiation is not a direct or purely circulatory effect but is due to reflex activity (105). It has again been demonstrated that it is possible to materially lower the blood pressure by carbon-arc irradiation (64).

Blood.—By the use of a lamp emitting parallel rays and with a distribution closely approximating sunlight, definite effects on hemoglobin content and on the number of red and of white cells have been obtained (89). Ultraviolet irradiations produced a temporary rise in the reticulocyte count in boys, ten to twelve years old (36). The influence of ultraviolet radiation on resistance to infection is controversial. Irradiation of the blood as it flowed through a quartz tube intercalated in the common carotid artery in rabbits injected intravenously with fatal doses of *Salmonella typhimurium* or *S. paratyphi*, resulted in a marked increase in survival time and in recovery in about 8 per cent (4). Ultraviolet irradiation decreased the death rate of streptococci infected mice (94).

Greater immunity to infection was assured by injection of a streptococcus vaccine prepared by ultraviolet irradiation than by one which had been heat-killed. The former appeared less toxic for intradermal tests and experimental animals showed less local redness and swelling (92). The Shope papilloma virus loses virulence but retains antigenicity under ultraviolet irradiation (137).

There are differences in the action of energy of various wave lengths on the coagulation time of plasma and of solutions of fibrinogen (101). For sources emitting much ultraviolet the coagulation time of fibrinogen increased and then decreased when the irradiation was longer than ten to twenty minutes. For plasma there was an upward trend too, but no reverse action, the longer the irradiation the greater the delay in coagulation. For sources emitting less ultraviolet and more luminous and infrared radiation, the coagulation time of fibrinogen was decreased when the exposure lasted ten minutes, with a distinct trend toward delayed coagulation when the irradiation lasted longer. For plasma, the coagulation time increased after an irradiation of from ten to twenty minutes, followed by a reversal in effect when the irradiation was prolonged. The probable explanation of these phenomena resides in differences in physicochemical state and chemical composition (101).

Metabolism.—Ultraviolet and luminous rays lower the blood sugar in normal men and in diabetics. The action is said to be similar to that of insulin, inasmuch as the glycogen content of the liver and muscle increases (100). Excessive doses of radiant energy cause a derangement of vitamin-C metabolism only to the extent that they are capable of producing injury (59). New evidence has been brought forward of a stimulating influence on the thyroid, its activity being increased as a result of the H-like substances produced in the skin (23, 24, 26). There is evidence that ultraviolet irradiation may increase the formation and excretion of androsterones (93). Exposure to artificial luminous and ultraviolet rays causes increased or accelerated gonadal activity in birds. Sparrows of both sexes when fed with wheat grains irradiated with ultraviolet rays also showed a marked acceleration in development. A chemical substance in the wheat was changed and when this was eaten directly it affected the anterior hypophysis which in turn stimulated gonad activity (96). In the assay of androgenic material light plays an important role as a modifying factor in the weight response of the chick's comb; the response is greater in birds receiving normal daylight than in those kept in the dark or in inadequate or subdued light. Body weights indicate a lack of growth which is in direct correlation to the deficiency of light (120).

An inverse relationship, not necessarily causal, is suggested between the caries-attack rate in white boys, twelve to fourteen years of age and the mean annual number of hours of sunshine (18). The quantum efficiencies of certain wave lengths of ultraviolet in the activation of 7-dehydrocholesterol have been measured and compared. Rats whose skins have been irradiated with ultraviolet corresponding to the dominant mercury lines in the region bounded by 253.7 and 302.5 $m\mu$ exhibit an antirachitic response, the potency at 296.7 $m\mu$ being significantly greater than that at other wave lengths. There is a similarity between the activation curve of 7-dehydrocholesterol and of rat skin whereas the activation of ergosterol is dissimilar. In all probability 7-dehydrocholesterol is the significant pro-vitamin D of the skin (12).

The respiration of rat skin irradiated *in vivo* by ultraviolet rays is stimulated; the effect is direct and is not due to consequent erythema (70). The oxygen uptake of rachitic rat skin is only 60 to 70 per cent of that of rats on the same basal diet, supplemented, however, with sufficient vitamin D to prevent rickets. As the rats

recover from rickets the skin respiration increases and reaches normal (102).

The division of sea urchin eggs is retarded when they are irradiated with large doses at 253.7 $m\mu$; following smaller doses the rate of cleavage is not influenced (33). Radiation from an intense mercury vapor lamp will activate the unfertilized eggs of *Arbacia punctulata*. The lowest effective energy is at 226 $m\mu$ (2.25 to 4.2 ergs per egg for 80 per cent activation); activation becomes negligible around 250 $m\mu$. Apparently a mechanism which starts with the absorption of the ultraviolet by nucleic acids is excluded (54). The radiation activates non-nucleate fractions and the effect therefore must be primarily upon the cytoplasm, with a secondary effect on the nucleus (47).

An effect of luminous and, to a lesser degree, of infrared radiation in promoting growth and accelerating development in rats has again been demonstrated. The rate of vitamin-A storage, with increased weight at given ages, is greater in rats deprived of than in those exposed to light (79).

Energy with the approximate spectral composition of sunlight promotes the germination of oats and barley, but a higher content of ultraviolet has an inhibiting effect (103). There is a change in the mineral composition of tomato plants when irradiated with a quartz mercury lamp. This is correlated with the level of calcium and phosphorus in the nutritive medium. The relationship between the two elements is reciprocal (121). Supplemental intermittent light of sufficient intensity and duration has a definite accelerating influence on the flowering of gladioli and carnations (3). There is a positive correlation between the growth of the gladiolus stem and temperature but a negative one with hours of sunshine. This latter negative effect is approximately four times greater than is observed with maize (81). Cowpea seedlings grown in light have an ascorbic acid content four to nine times that of those grown in darkness. The greater production in light is due to the activity of the chloroplasts (104). Manchu soy beans, grown on a substrate deficient in nitrogen and dependent on fixation of atmospheric nitrogen, often show nitrogen hunger during growth. This occurs under conditions favoring photosynthesis and all the signs of carbohydrate excess are present. The carbohydrate excess inhibits nitrogen fixation and removal of the plants to the shade at the stage when nitrogen hunger occurs is followed by rapid and normal

development (30). The quantum efficiency of photosynthesis in *Chlorella* is of the order of 0.05 to 0.08 mols per quantum, much lower than the older value of 0.25 (83, 97).

Photodynamic sensitization.—Photodynamic action depends on the concentration of molecular oxygen as does the influence of sensitizer (hematoporphyrin) concentration on the photooxidation of activated acceptor. The proteins of blood and lymph and the uric acid of urine account for the photooxidation of blood, lymph, and urine in the presence of the sensitizer. The extent of the photooxidation depends on various factors, such as intensity and absorption of light, length of exposure, concentration of acceptor and of sensitizer, temperature, pH, and partial pressure of oxygen. The general effects of photosensitization in the living animal, due to oxidation of plasma acceptors, depends on the size of the surface area irradiated and are due to the consequences of this primarily local damage (116).

Severe cutaneous and general reactions (elevation of temperature, accelerated pulse) have been reported following exposure to light during or following the administration of the sulfanilamide group of drugs (29, 38, 84, 108). These drugs, however, are not photodynamic sensitizers (11, 21). Perhaps they render the skin hypersensitive to the action of normal erythema. An estrogenic deficiency in females suffering from photogenic dermatitis has been suggested (68). Luminous rays liberate histamine from perfused lungs in the presence of hematoporphyrin (61).

No significant effect is exerted on the oxygen consumption of animal organs through feeding with buckwheat. If a photosensitizing substance exists in such animals its concentration is too low to affect the oxygen consumption, and such a photodynamic substance has not yet been demonstrated in buckwheat disease. Perhaps there is a change in the tegument of the animal, rendering the skin more susceptible to irradiation (117). Irradiation with luminous rays influences the excretion of coproporphyrin; the effect is strongest in red light (37). The administration of nicotinic acid amide to pellagrins does not influence the excretion of porphyrins but does lower the sensitivity to radiation. The presence of nicotinic acid amide may be essential as protection against the precipitating action of sunlight in producing skin manifestations (67). The relation of the porphyrins to light sensitivity has been reviewed recently with emphasis on the fact that the different types

and kinds of porphyrins vary considerably in their photosensitizing action (16).

The percentage curve for photodynamic hemolysis shows that the process is composed of a photochemical reaction and an effect independent of light. The photoprocess, irreversible and additive, dominates the curve so that any point on it up to and beyond 50 per cent hemolysis may be used as a measure of effectiveness (9); it follows the reciprocity law (10). The number of quanta required for lysis of the red cell is independent of the dye concentration. An important limiting factor in photodynamic hemolysis is the extent to which the dye is taken up. The number of quanta absorbed by a dye molecule in this process is inversely proportional to the concentration and ranges from a few to thousands of quanta. The dye must act in a cyclic process, probably transferring its energy to substrate which then reacts with oxygen, the dye returning to its normal state (10).

The apparent resemblance between the effectiveness of the wave length region between 290 and 300 $m\mu$ for the production of erythema and of neoplasia is of great interest (88). Carcinogenesis by ultraviolet irradiation is usually preceded by repeated severe erythematous reactions. The relationship to erythemogenesis may signify that the production of neoplasia is related to chronic inflammatory and atrophic changes. There is the possibility that carcinogenic agents may be liberated from proteins as well as from sterols.

Ultraviolet irradiation produces skin cancer in man, possibly by increasing an already present predisposition, causing a tumor to appear earlier and become more malignant. Substances activated by sunlight are absorbed and transported throughout the body. Even if their concentration is not sufficient to produce tumors they may enhance a predisposition. While the amount of ultraviolet radiation ordinarily used in therapy is far below the carcinogenic dose, it should be emphasized that it is dangerous to regularly and strongly irradiate the entire surface of the body with strong sunlight (58).

The theory of the developmental physiology of malignant tumors has recently received some support. The cancer cell is always present, needing only a "realization factor" in order to develop into a malignant tumor (23). Tumor cells from Ehrlich's mouse carcinoma implanted into mice have had their development definitely influenced by chronic irradiation of relatively short duration

and small dose, approximately one to two times the erythema threshold. Perhaps this is accomplished by the setting free of H-like substances, or of an increased amount of thyroxine, or by the accumulation of cholesterol (25).

The rate of tumor production by ultraviolet radiation varies with time, the amount of pigment and the character of the diet. When the effect of various substances on the development of tumors by ultraviolet radiation was studied by applying them directly to tissues in which neoplastic changes were occurring it was found that the rate of tumor production by ultraviolet radiation could be altered by local application. Of oils, mineral oil accelerated tumor development most rapidly, cottonseed, olive, and wheat germ oil caused slight acceleration and linseed oil retarded. Cholesterol in oil caused marked acceleration. Peroxides and 1,2,5,6-dibenzanthracene had no effect (112). Cholesterol in the diet fails to stimulate the production of tumors in the rabbit by ultraviolet irradiation. This, as well as other evidence on the failure of cholesterol to influence the development of tumors leads to the conclusion that it is doubtful whether cholesterol is a tumor-promoting agent (6).

The conception that actinic or sun cancer is caused by some interaction between the ultraviolet solar rays and the cholesterol present in the skin (109) has been subjected to criticism and study (7). The claim is made that cholesterol is a heliotropic substance which migrates to those parts of the skin subjected to sunlight and is an accumulator of actinic energy. Under the influence of ultraviolet rays and the accompanying oxidation, the sterol loses its characteristic chemical reactions and acquires new properties such as ionization, fluorescence, luminescence, radiation, and photoactivity. Cholesterol is photooxidized by ultraviolet radiation in the presence of air. It loses the side chain, undergoes esterification of the tetracyclic ring system and is finally dehydrogenated to a carcinogen resembling derivatives of the phenanthrene series. In the development of this conception of the etiology of actinic cancer, the observation was made that the cholesterol content of the skin of the exposed parts of the body was from three to six times higher than that of nonexposed parts. Also when rats were irradiated with sunlight or with the energy of a mercury vapor lamp the cholesterol content of the skin was increased by 23 to 100 per cent. This

has been confirmed, with the interesting observation that the cholesterol is largely in the ester form (63).

There are, however, numerous observations which make it appear unlikely that cholesterol acquires photoactivity upon irradiation but rather that the phenomenon observed is due to the chemical action of peroxides. Likewise, it appears unlikely that the photooxidation products of cholesterol are dehydrogenated in some manner to give rise to substances related to the carcinogens of the phenanthrene series. Also, there is little, if any, evidence that cholesterol becomes carcinogenic on irradiation. The data obtained from a study of the effects of applying irradiated cholesterol to the skin of mice leads to the conclusion that irradiated cholesterol does not act as a carcinogen (7).

Coal tar derivatives are much more efficient than wood tar as sensitizers. They lower the erythema threshold and cause increased reactions. The wave lengths involved are between 320 and 405 $m\mu$. The tar is absorbed by the skin; hence the wave lengths it absorbs are added to those usually effective in producing erythema; thus the skin is endowed with greater reactivity to longer wave lengths (98).

There may be some connection between carcinogenic and estrogenic substances, because burning, radium and ultraviolet irradiation, all of which may produce neoplasia, may also lead to the appearance of folliculin-like substances (28). The urine of normal mice exhibits no photodynamic action nor does urine from animals inoculated with hydrocarbons which do not produce tumors; but when the mice are inoculated with tumor-producing hydrocarbons the urine always contains photodynamic substances (91). A comparison of the photodynamic activity of some carcinogenic and noncarcinogenic compounds shows that the latter are less potent photodynamically than the former (17). The presence of 3,4-benzpyrene has been detected, by its photodynamic activity on *Paramecia*, in the lungs and liver of mice painted or inoculated with 3,4-benzpyrene (91). A destructive effect occurs if yeast is exposed to radiation of wave lengths 345 to 450 $m\mu$ in methylcholanthrene suspension. This is essentially a photochemical process (photodynamic) (56).

Xeroderma pigmentosum is the most characteristic example of an actinic dermatosis which may give rise to cancerous transforma-

tion. It is due to an abnormal reaction of the skin to sunlight, generally manifested in early infancy (22). Photosensitization would seem to be a feature of lupus erythematosus rather than its cause. Dissemination not infrequently follows exposure to sunlight or may be a result of treatment with ultraviolet radiation (90).

Bacteria and other fungi.—Interest continues in the sterilization of air in hospital wards and operating rooms by means of radiation (43, 45, 46, 65, 82, 106, 107, 115, 125 to 130). The physical factors concerned in bactericidal irradiation of air have been set forth in detail (128), as well as the bacteriologic and epidemiologic factors in infection of air (126). Air is an important source of contamination in every operative wound and sterilization of the air can be accomplished by bactericidal irradiation. The majority of the pathogenic bacteria are staphylococci which cause by far the largest number (over 90 per cent) of wound infections. Postoperative wound infections have been reduced more than 85 per cent, as well as the incidence of postoperative pyrexia (44). Wounds exposed to an appropriate intensity of radiation, heal with less reaction, less risk of infection and with less systemic reaction (45). Barriers of ultraviolet rays are effective in preventing the spread of infection in an isolation ward and of artificially introduced bacteria from cubicle to cubicle. The effect is great when air movement is slow and less so when rapid. Either air-conditioning or ultraviolet lamps markedly reduce the number of air-borne bacteria; ultraviolet is the more effective (106, 107).

The study of the effects of sublethal doses of monochromatic ultraviolet radiation, mostly 265 m μ , on the growth properties of bacteria show that, in general, colonies formed by bacteria which survive irradiation appear somewhat later than colonies formed by the controls: there is a noticeable lag in development, an extended lag phase, but, when the growth curves are followed for a few hours, a second effect becomes apparent, namely, the numbers apparently increase considerably during the first hour or two of incubation, provided that the bacteria have received such quantities of radiation as to yield a fairly low survival, a high percentage killing. Evidence seems to point to the hypothesis that the "apparent initial increase" is a multiplication of cells. There is also the probability that over and above the intracellular products that may determine the normal lag there is doubtless a further addition of inhibiting substances as a result of irradiation (55).

Inactivation data on spores of *Trichophyton mentagrophytes* exposed to measured quantities of monochromatic ultraviolet radiation indicate that 253.7 to 265 $m\mu$ is the most effective region. It takes approximately 7×10^{-4} ergs to obtain 50 per cent inactivation in these spores as compared with 8×10^{-6} ergs in bacteria (57). In addition to the lethal effect there are others, the most striking being mutant production, which is also greater after exposure in the region between 253.7 and 265 $m\mu$. The rate of mutation reaches a maximum, in the particular strain used, at 265 $m\mu$ at an energy level of 100×10^{-4} ergs per spore and rapidly decreases with increasing amounts of energy. Ultraviolet irradiation apparently accelerates the normal rate of mutation. The fact that nucleic acids have their most intense absorption band in this same region is significant (27).

The most effective wave length for the inactivation of yeast is around 260 $m\mu$ and some 500 ergs per sq. mm. produces 50 per cent inactivation. The ultraviolet absorption spectrum of *S. ellipsoideus* exhibits a maximum at 265 $m\mu$; the extinction coefficient decreases for inactive cells and reveals a secondary maximum at 280 $m\mu$. Dividing cells show increased nucleic acid metabolism (69).

Since the killing of living organisms by heat is accompanied by coagulation of protoplasm it is reasonable to suppose that their heat resistance would be decreased by ultraviolet irradiation. This was demonstrated to be true for the spores of three cultures, *Bacillus cohaerens*, *B. albolactis*, and CC. The spores most easily sensitized are those of high heat resistance. The change produced is apparently irreversible since the tolerance to heat was found to be independent of the time which elapsed between the two treatments (15). Ultraviolet radiation (253.7 $m\mu$) may be so applied to tubercle bacilli that they are rendered nonvirulent without being made nonviable, and the irradiated viable tubercle bacilli may induce demonstrable immunity in experimental animals. Organisms killed by the radiation do not induce measurable immunity (118).

The deleterious effect of the photodynamic action of dyes on antibodies has been demonstrated, but the antibody protein remains completely soluble in saline and its relative viscosity is not appreciably changed (138). Several dyes are lethal for gram-positive organisms but not for gram-negative. Safranin is, however, along with low native bactericidal power. Hydrogen peroxide enhances its photosensitizing action (124). Methylene blue in the

presence of light destroys antipneumococcal serum. Neutralization of diphtheria toxin by antitoxin is inhibited after an exposure of a mixture of antitoxin and methylene blue to light (110). Erythrosin and dizyanin-A sensitize bacteria to long wave luminous energy and to the infrared (75).

Protozoa.—Each species of a number of protozoans was found to have a characteristic resistance to ultraviolet radiation (253.7 $m\mu$). The most susceptible form extinguished the most energy per unit area. Changes in resistance were due primarily to altered physiological state and secondly to small variations in environment (34). Wave lengths of 313 and 366 $m\mu$ had no visible effect in large doses on protozoa but when the dosage with 313 $m\mu$ was sufficiently great the encystment time of *Colpoda* was increased (35). Cell division in *Paramecia* was more retarded by irradiation at 280.4 $m\mu$ than at 265.4, but recovery was more rapid, presumably due to the fact that the injury at 280.4 was localized in the cytoplasm which was more readily repaired than was injury to the nucleus by irradiation at 265.4 $m\mu$ (34).

Proteins.—Secondary protein derivatives, which can be dialyzed at pH 4.8, are formed by the ultraviolet irradiation of egg albumin at 25°C. They are probably the result of a breaking of the polypeptide chain. The weights of ovalbumin precipitated at the isoelectric point after irradiation indicate that the formation of denatured protein, and of soluble protein derivatives from the denatured protein, are first-order processes. Oxygen does not influence the denaturation process but increases the formation of soluble protein derivatives. Denaturation and molecular splitting are due to different processes (8). Evidence suggests that ultraviolet irradiation and soft x-rays cause liberation of material of low molecular weight which, together with albumin residues, undergo photooxidation reactions which result in increased light absorption (114). The ultraviolet absorption spectra of several purified plant viruses indicate that they contain nucleic acid (74).

Mode of action.—The underlying mechanisms of the effects of radiation are obviously quite different from those observed and the fundamental processes are in great measure unknown. On what part of the living cell does the radiation act and what is the primary change? How does the observed final action follow the primary effect (136)?

The setting free or the elaboration of histamine or of H-sub-

stances is thought to be involved in many effects (23, 25, 64). Radiations produce colloid chemical changes, in living cells as in proteins—aggregation, change in solubility, increased viscosity, and coagulation. Increase in fluorescence, as a result of the appearance of photooxidation products, indicates that the primary effect is a structural and chemical change. The increase in fluorescence is proportional to the amount of available oxygen (131). Final coagulation is the fundamental process in the inhibition of cell division caused by irradiation. An increase in fluorescence occurs in the nucleolus and in the network of the nucleus. There is something here that is fundamental to the production of mutations (132). In the photochemical changes indicated by increase in fluorescence we have the primary effect of radiation; coagulation follows when the photochemical change reaches such a degree that the solubility properties of the protein constituents change. The change in chemical structure is intramolecular, following immediately on absorption by individual molecules and is independent of environment. Flocculation, which follows the chemical change, is dependent on environment (134).

Catalytic effects of radiation are concerned with reduction processes. The substances whose reduction is catalyzed by radiation are sulfhydryl in character; they occur in considerable amounts in the germinal skin layer, but not at all in the horny layer because the sulfhydryl groups have been here oxidized to the disulfide. These sulfhydryl groups account for the strong reducing power of the skin. Shorter ultraviolet wave lengths are more effective than long and these in turn are more effective than luminous rays (95, 134). Sulfhydryl groups are important for cell respiration, the elaboration of antitoxins and in detoxication. The intrinsic protective and healing processes of the skin are based on its richness in sulfhydryl groups. This is the function of the rich supply of sulfur in the skin. The sulfur is concentrated in the form of sulfhydryl groups, accessible to the body in fluid exchange and accessible to the action of radiant energy; thus, owing to the catalytic action of the latter, they carry out important functions with greatly increased velocity (134). Radiation may not only catalyze the reducing action of sulfhydryl groups already present but also form new sulfhydryl groups; thus, the photochemical formation of active substances, such as vitamin D, sex and adrenocortical hormones, the carcinogens, aldehydes, and adenylic acid (133). Sulf-

hydriyl groupings enter into antigenic specificity and enzyme destruction by ultraviolet energy (99).

Here, as in so many instances, the observed facts and their application in explaining others have far outstripped our understanding of the mechanisms by which the observed facts or findings are obtained. In irradiated tissues atoms and molecules are ionized or activated. These may lead to chemical, colloidal, changes in the molecules and thus cause the observed effects. It has recently been argued that the primary process consists in changes in or destruction of controlling centers, or regions of greater sensitivity, e.g., nuclei. The development of this concept of action on a controlling center has led to a so-called Impact (Treffer) Theory, based on the quantal nature of radiation (136). Manifest effects are due to "mutations" in the individual cells, resulting from the absorption of quanta of energy by the chromosomes or genes. These mutations give rise to the variety of observed effects.

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PHYSIOLOGICAL ASPECTS OF GENETICS

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There is scarcely a branch of genetics that is not of potential physiological interest; on the other hand, it often happens that as physiological aspects are more emphasized in a given field, the work comes to seem less reasonably included under the heading of genetics. For these reasons any such review as the present one must be more or less arbitrarily limited. In the present case several subjects have been excluded in part because satisfactory recent reviews already exist. Examples are the genetics and chemistry of flower colors (Lawrence & Price), the genetics of lower plants and work on viruses (Stakman *et al.*; Christensen & Rodenhiser), and the newer studies of the effects of irradiation on mutation and on chromosome rearrangements [Bauer; Muller (1, 2); Timofeeff-Resovsky & Zimmer; Stubbe].

Attention may also be called to four books covering the general subject of genetics—new editions of the well-known texts of Sinnott & Dunn and of Snyder, and new general treatments by Waddington (2) and by Sturtevant & Beadle.

SEX IN ALGAE

Moewus (1, 2, 3, 4) and Kuhn, Moewus & Wendt have reported a series of experiments with various races of the unicellular alga *Chlamydomonas*. A series of carotenoid substances (crocin, *cis*- and *trans*-crocetindimethylester, safranal, picrocrocin) are reported to have quite striking and specific effects on the sexual reactions of the alga. All are apparently produced by *Chlamydomonas* (except picrocrocin, which is represented by a closely similar substance) and all are likewise extracted from the styles of *Crocus*. The suggestion is made that they are also involved in the sexual differentiation and reactions of the tissues of seed plants. These results have been criticized by Philip & Haldane, Pringsheim & Ondracek, and others. There are so many serious doubts about the work that the reviewer does not feel justified in discussing it in detail. However, if the results are confirmed they will constitute a great advance in

the understanding of the inheritance and physiology of the functions concerned.

MATING TYPES IN INFUSORIA

Work on the genetics and physiology of the mating reactions in *Paramecium* has continued. Sonneborn (1, 2) shows that in one strain of *P. aurelia*, there is a single pair of genes, in the micronucleus, that controls the mating type. All aa individuals are of Type I; Aa or AA are of either Type I or Type II. "Endomixis" is accompanied by a reorganization of the macronucleus, and the mating type is controlled by the composition of the macronucleus. In clones carrying A (either AA or Aa) the new macronucleus may be of either the Type I or Type II composition. The nature of this relation is not clear, but the evidence is convincing that such are the facts. The results also show that, in this strain at least, "endomixis" results in the establishment of homozygosis in the micronucleus, thus confirming the description of "autogamy" by Diller. It is possible that this may not apply to other strains, which certainly react differently in some respects, but for which no extensive experiments have been recorded.

Mating types more or less similar to these have been described and studied in *P. bursaria*, *P. caudatum*, four other species of *Paramecium*, and in *Euplotes* [Jennings (1, 2); Chen (1, 2); Sonneborn (1, 2); Gilman; Kimball; Tartar & Chen]. Many interesting phenomena are recorded in these papers, which constitute the framework of a new and powerful method of studying the genetics of the Infusoria.

Only a few of these results can be briefly mentioned here. Some clones of *P. aurelia* produce toxins that are lethal to certain other clones; the property of producing such toxins is inherited. In several of the species concerned (all that have been studied extensively) there are several indistinguishable but wholly cross-sterile groups of mating types. In several such groups (or "varieties") there are four, six, or eight different mating types. Individuals of any one type will conjugate with those of any one of the others—i.e., if one wishes to compare the mating type situation with sexual differentiation, there are four, six, or eight "sexes." The conjugation itself is independent of polyploidy, occurs with cell fragments, and at least in one "variety" is inhibited by continuous light. In *P. aurelia* the mating reaction and the associated specificity develop

within a week or less, in *P. bursaria* the period required for the development of "sexual maturity" may be over 6 months. In *Euplotes* no agglutination occurs, but the first visible reaction is a fusion of the animals in pairs. In *Euplotes* also, though not in *Paramecium*, conjugation is stimulated by cell-free medium in which a given type has been grown; addition of such cell-free medium may cause conjugation within a clone of a different mating type.

SEX DETERMINATION IN HYMENOPTERA

The work of Bostian and of Whiting (1, 2) has cleared up some of the long-standing problems in the inheritance of sex in the Hymenoptera. It is now shown that, in *Habrobracon*, there is a series of alleles, of such a nature that an individual carrying any one (either haploid, or diploid and homozygous) is male, while an individual carrying any two different alleles is female. The number of such alleles is apparently large; only three were present in Bostian's experiments, as a result of the design of the experiments, but Whiting's analysis of other data makes it probable that many exist. No physiological interpretation of these relations has been presented, but the difficulty of forming any picture of a possible developmental mechanism suggests that there is here a problem of real physiological interest. It is possible to suppose that two alleles exist, each of which produces a characteristic substance not produced by the other, and that femaleness results from the interaction of these substances; but how, then, is one to picture the action of a third allele complementary to each of these? The existence of a still larger series of mutually complementary alleles seems to require the framing of some hypothesis different in kind from those usually postulated in connection with gene action.

POLYPLOIDY AND SEX IN HIGHER PLANTS

The use of colchicine and of acenaphthene to influence mitosis, and in particular the induction of polyploidy by colchicine, has led to so many papers that a summary here is out of the question—even Sunday newspaper supplements have had many accounts of the work. The general method is, however, of promise in connection with many problems of physiological interest. One example may be cited. In *Melandrium dioicum*, a seed plant, it has long been known that sex determination is similar to that in many

animals, XX being female, XY male. In *Drosophila* it has been shown that the autosomes are male-producing in their net effect, the X, female-producing, and the Y practically negligible. Using colchicine-induced polyploids, Warmke & Blakeslee (1, 2) have now shown that the system is different in *Melandrium*, the Y being male-producing and the autosomes having little or no effect. One result is that a fertile dioecious tetraploid strain may be produced; by analogy with *Drosophila* it had previously been supposed that this would be impossible.

SELF-STERILITY

The phenomenon of self-sterility has seemed to offer interesting possibilities for the study of the interrelations of genetic and physiological processes ever since the discovery of the "oppositional factor" system by East & Mangelsdorf in 1925. In many self-sterile plants there exists a series of alleles, of such a nature that pollen tubes carrying a given allele will not grow well in a style carrying that same allele. East's catalogue of the distribution of self-sterility among seed plants (including many new observations) has now been published posthumously (see also a general review by Kuhn). Emerson (1, 2) has reported new observations on the physiology of the reaction in *Oenothera organensis*. Grafts, both of styles and of roots, have yielded no evidence for any diffusible substance concerned; the properties of each region of the style are determined by the genetic composition of the region itself. The inhibition found in incompatible pollinations may largely disappear in the styles of plants kept in the dark for three to six days. Brink & Cooper (1, 2) have reported a new type of self-sterility, in alfalfa. They find that incompatible pollen tubes, though they grow less rapidly than compatible ones, often accomplish fertilization; but the embryos arising from selfing abort much more often than those resulting from cross-pollination. Histological studies lead to the interpretation that fertilization results in stimulating growth, both of the embryo sac (chiefly the endosperm at first), and of the surrounding tissue. There is a competition for available food reserves, and unless the endosperm grows rapidly the surrounding maternal tissues hypertrophy sufficiently to starve the embryo. The growth rate of the endosperm is presumably stimulated by heterosis in cross-pollinated seeds, and usually not adequately so in selfed ones.

ERYTHROCYTE SPECIFICITY

Boyd and Wiener have published summaries of the existing data on the blood groups of man. Irwin and Irwin & Cole have continued their studies on the genetics of erythrocyte antigens in dove species hybrids. When Pearlneck and Ring Dove are crossed, the hybrid (as previously reported by these authors) contains most or all of the antigens of both parent species. On repeated backcrossing it has been possible to segregate at least ten antigenic groups, present in Pearlneck but not in Ring Dove, that now behave as though dependent each on a single gene or group of linked genes. Less complete data show that there are at least nine such separable antigens present in Ring Dove and not in Pearlneck, and at least three present in Pearlneck and not in Senegal. Senegal contains all or nearly all of eight of the specific Pearlneck (not Ring Dove) characters, and only part of the remaining two. The F_1 from Ring Dove X Pearlneck contains one, or more likely several, "hybrid substances," not present in either parent; these are found, in the backcross birds, to be definitely associated with the presence of some but not of all the species-specific substances.

LEUKEMIA IN MICE

MacDowell has summarized the earlier work on his leukemic strain of mice. The mice of this strain usually die of a spontaneous leukemia, and such a spontaneous leukemia may be transmitted to other mice of the same strain by inoculation of a large number of leukemic cells. Most of the results obtained with transplantable tumors may be paralleled; in fact it is clear that the condition is best considered as a cancerous condition of the leucocytes, with its special peculiarities due merely to the fact that the proliferating cells are not organized into a coherent tissue but are free in the blood stream. If a line of leukemic cells is passed successively through a long series of hosts its virulence (measured by the minimum lethal dose and by the time required for a given dose to kill the host) increases markedly. If a susceptible mouse is given sublethal inoculations, immunity to the particular line of leukemic cells used may be induced. (There are also other ways of conferring a partial immunity on susceptible mice.) MacDowell, Potter & Taylor show that mice completely immunized to an old virulent line of leukemic cells are not thereby protected from death due to a

new spontaneous leukemia, nor are they immune to leukemic cells from a newly arisen case of leukemia. There is, however, some cross-immunity between different old virulent lines, even (in one case tested) when these lines originally arose in different strains of mice. Evidently there is a difference in the antigenic content of old and of new leukemic lines, the old lines containing one or more effective antigens that are not present or do not react in the newly arisen lines.

TUMORS IN HYBRID MICE

Little has recorded a new observation on the inheritance of rate of production of spontaneous tumors—namely, that the first generation hybrids between ordinary mice (*Mus musculus*) and the related species *M. bactrianus* have a much higher incidence of spontaneous tumors than either parental race. Many workers have studied the rates in interspecific hybrids in mice, and have found no such relations. It remains uncertain what interpretation is to be put on the new result. Little suggests that it is related to physiological disharmony between the two parental sets of genes. The reviewer would suggest that, on the mutation interpretation of the origin of tumors, the high degree of heterozygosis of the hybrids may make the appearance of recessive somatic mutant areas more probable.

It is possible also that the result may be correlated with the observation by Sturtevant that the crossing of the two semicross-sterile races of *Drosophila pseudoobscura* leads to a greatly increased mutation rate.

TUMORS IN DROSOPHILA

Russell has studied the tumors of *Drosophila*. She finds that those examined are benign, in the sense that no unfavorable effect on the host can be attributed to them. Groups of cells free in the body cavity of the larva become covered with a melanotic capsule, and it is this visible sign that has led to the detection of the condition. The bodies proliferate, increasing both in number and in size, during the larval stage. This happens even when tumors are transplanted to animals of strains in which no tumors occur spontaneously. With pupation of the animal, growth of the tumors ceases, the cellular contents disintegrate, and the melanin deposit is increased. The lethal effect of the l-7 gene is shown not to be due to

to the tumors associated with it, but probably to result from a defect in the gut that interrupts the continuity of its lumen.

TRANSPLANTATION AND CHROMATOPHORES

The technique of transplanting tissue to chick embryos has yielded evidence on the manner of action of the melanophores, the properties of which are under genetic control. Most such experiments have been made with intraspecific interbreed transplants, but Eastlick (1) has also studied intraspecific transplants. Even duck leg-buds transplanted to chick embryos may produce typical and well developed donor limbs; but shortly after hatching these heterospecific grafts regularly degenerate and are absorbed—evidently as a result of an immunological reaction on the part of the host. This result is of interest in connection with the studies on pigmentation in embryonic grafts [Dorris; Eastlick (1, 2, 3); Rawles; Willier & Rawles]. These experiments show that the melanophores are derived from neural crest tissue, and migrate to the other parts of the embryo. Their source is different from that of the follicle cells, and thus it happens that feathers of host structure may have donor melanophores. Under these conditions the donor melanophores develop according to their own constitution, even in such a "wide" transplant as robin to chick (Rawles). However, the donor melanophores are gradually replaced by host ones, and the final plumage of the operated bird shows no effects of the graft, again evidently because of an immunological reaction.

The results of Twitty & Bodenstein and of DuShane on grafts in salamander embryos (*Triturus* species and species-hybrids, axolotl color variants) have shown that here also the melanophores are of neural crest origin and that the donor melanophores develop according to their own genetic constitution.

Reed has made similar studies with rodents. He has drawn an interesting distinction between the determination of the properties of a given area and of the individual cells within such an area. Some of his results are interpreted as meaning that there is an influence of surrounding areas on the structure of the hair, in the sense that the recessive "waved 2" of the mouse may impress the waved character on genetically not-waved adjacent tissue. The results are complicated by a rather rapid invasion of donor tissue by host tissue. Reed assumes that the pigment is a clear index of the origin of the hair (from host or from donor); to the reviewer

the results are so suggestive of those found with birds as to indicate strong possibility that here again the pigment is due to melanophores arising outside of the follicle, a view that has been held by some histologists.

EYE-COLOR HORMONES IN INSECTS

The study of the substance (or substances) found in various insects that is capable of changing the "a" mutant eye-color of *Ephesia* into wild-type ("a⁺ hormone") or the vermilion-eyed mutant type of *Drosophila* into wild-type ("v⁺ substance") led Khouvine, Ephrussi & Chevais to suggest a relation between hormone production and tryptophane. Tatum then found that a certain strain of bacteria was able to synthesize a substance having the properties of the hormone, when grown on a medium containing tryptophane. This bacterially produced substance was obtained in crystalline form by Tatum & Beadle (2). From these facts, together with the data obtained by the above authors and others on the chemical properties of the hormone, Butenandt, Weidel & Becker were led to try the effectiveness of various tryptophane derivatives, and found that the effects on *Ephesia* and on *Drosophila* could both be produced by kynurenine. It appears that this substance is either identical with that produced by the insects themselves, or is closely related to the naturally produced hormone. It may be expected that the physiology of the production and functioning of the hormone, about which much is already known [see, for example, deMello; Ranzi; Clancy; Beadle, Tatum & Clancy; Tatum & Beadle (1)], will now be more easily and effectively studied. Since the genetic control of hormone production is a simple one, one may hope that these studies will throw light on the physiology of gene action.

Here, as in most such studies, a theoretical difficulty arises. The usual technique is to study the development of some character known to be under genetic control, and to determine the nature of the earliest detectable effect of the gene in question. There is no *a priori* reason why the method may not, in certain cases, lead to a knowledge of the primary effect of a given gene; the difficulty is that, at present, there appears to be no way of deciding the point in any given case. That is to say, the chain of developmental reactions may be traced back to the gene, but there is no way of determining when one has reached the gene.

COMPLEX MUTANT TYPES IN VERTEBRATES

Grüneberg (1) has suggested one possible index here. His detailed studies of several mammalian mutant types [Grüneberg (1, 2); Beer & Grüneberg; Bourne & Grüneberg; Engel & Grüneberg; Grüneberg, Hallpike & Ledoux; Grüneberg & Lea; Fell & Grüneberg—compare also the work of Landauer (1, 2) on fowls, and of Smith & Bogart on rats] have shown that phenotypically complex types can sometimes be interpreted as dependent on a single primary effect, the complex of observed symptoms being secondary consequences. Grüneberg has further observed that these secondary effects are more variable in their incidence and in their degree of manifestation than is the primary effect. He, therefore, suggests that the primary gene effect may be, in general, more constant in its manifestation than are secondary effects. While the conclusion may be granted as probable, it does not appear to the reviewer to be a safe or adequate criterion for application in individual examples.

MULTIPLE EFFECTS OF GENES

There is another implication in this analysis that is of physiological interest, namely, that a single gene has one or a few specific primary effects on development rather than a series of diverse effects produced at different times and places in the developing individual. This long-discussed problem has been studied recently by Schwab, who has attempted to determine the effects of individual gene differences in *Drosophila* on a single arbitrarily chosen character, the shape of the spermatheca. Owing to the inherent impossibility of obtaining animals known to differ only in single genes, the results are not unambiguous, but Schwab concludes "... for the ... proposition ... that many genes affect a multiplicity of characters, these data offer no evidence"—though it is clear also that the opposite conclusion is not supported. It may be pointed out, however, that there is a marked and growing tendency among geneticists to think in terms of single primary effects of genes.

GENES AND DEVELOPMENT

Waddington (3) has recently summarized the work on the relation between genes and development. This book is not only a summary of the earlier work, but also contains much new data,

and is an attempt to correlate the modern organizer concept of embryologists with genetic data and thereby to produce a general theory of development.

Goldfish.—Certain specific results in this field may be mentioned. Goodrich & Anderson have studied a one-gene difference in goldfish. In the wild type the chromatophores steadily increase in number as development proceeds; in the homozygous mutant type this increase ceases at about one week of age, and chromatophore degeneration sets in. In the heterozygote there are irregularly distributed areas of increase and of degeneration, as though the two opposing processes were nearly at equilibrium, and slight external influences easily shifted the balance. This is in agreement with the observation by Goodrich & Trinkaus that ultraviolet irradiation accelerates chromatophore production in the heterozygotes but not in either homozygous type. The authors suggest that the chromatophore destruction is probably under endocrine control.

Development of wings in Drosophila.—A detailed study of the development of the wings of *Drosophila* has been made by Waddington (1, 3), who studied the wild type and also a large number of mutant types. The mutant characters are related to specific differences in development, and are classified according to the time at which they are first identifiable and according to the nature of the first observable difference. There results a picture of the wing as a complex structure, resulting from a series of interrelated processes that are separately capable of modification by genes. Much of this picture is confirmed by the work of Braun (2), the chief point of disagreement being that Braun agrees with the earlier account (Goldschmidt) of a progressive degeneration of the wing margin in those mutant types that have the appearance of normal wings from which pieces have been cut away. No such degeneration is found by Waddington, who supposes that its description arose from a confusion of the sequence of events in wing development. In this connection mention may be made also of the study by Volovik of the effects of combinations of mutant genes affecting the wings.

Antennae of Drosophila.—Waddington (3) and Braun (1) have carried out, independently, a series of studies on the interaction of mutant types of *Drosophila* differing from the wild-type in leg and antennae characters; unpublished independent observations by the

reviewer confirm their most essential common results. The point of departure here is the long-known type *aristapedia*, in which the antenna has become quite leg-like—particularly the normally highly modified terminal segments (the arista), which are closely similar (in *aristapedia* flies) to tarsal segments. When *aristapedia* is combined with genes whose chief effect is on the arista and not on the legs of wild-type, there is no effect on the tarsus-like terminal antennal segments; when *aristapedia* is combined with genes whose normal effect is on the tarsi and not on the antennae, the characteristic effects of such genes are also observed on the antennal tarsus-like segments. Evidently *aristapedia* not only makes the tissue develop into tarsus instead of arista, but thereby also makes a corresponding change in its reaction to other genes.

Development of deficiencies in Drosophila.—Poulson has studied the developmental history of various *Drosophila* types in which certain chromosomes or sections of chromosomes are wholly absent, in this way determining the effects of known series of genes. All of these deficiencies are lethal—i.e., the chromosome sections studied are all necessary for the completion of development. Absence of the entire X chromosome leads to abnormalities in late cleavage stages, and no blastoderm is formed. Absence of the left half of the X results in an incomplete blastoderm; of the right half, in failure to separate the germ layers. Absence of the "Notch" region (a small portion of the left half of the X) results in abnormalities (nervous system, gut, absence of mesodermal organs) that are discernible only at stages never reached at all by the longer deficiencies.

Effects of external agents on development of Drosophila.—Several recent studies are concerned with the relation between the conditions of development (usually temperature or nutritional differences are studied) and the phenotype of the resulting individuals. A number of these papers concern the effects of sublethal temperatures; as has long been known, these often give rise to striking aberrations, which may resemble known mutant types. They have been called "phenocopies" by Goldschmidt, and this term is often loosely used in current literature. The reviewer would like to point out that it is difficult to imagine a viable aberration that is not rather similar to some known mutant type; while many well-known mutant types (e.g., the more extreme eye colors) have not been produced by external agents.

Child, Blanc & Plough have studied the effects of extreme high temperature on the phenotypes of flies heterozygous for a number of recessive genes. They conclude that in a number of cases the treatment leads to a "reversal of dominance." Blanc & Child have studied one of these examples (dumpy) in more detail, and have determined that it has a "temperature effective period" at about eight to ten hours after pupation for one effect ("vortex"), and at about twelve to sixteen hours for another ("truncate"). The data in these two papers do not seem to the reviewer adequate to establish that the effects studied are due to the particular genes to which they are attributed.

Temperature effects in *Drosophila* have also been studied for bristle types by Ives, Child (2), and Neel (1). The latter concludes that, in some cases at any rate, the observed effects of temperature on bristle number are secondary, the primary effects being on the size of the animal, which in turn affects the bristle number. Neel (2) has also studied the size of supernumerary bristles in relation to their position on the animal, and finds that the nearer they lie to the position of a normal bristle, the larger they are.

Child (1) has studied the effects of starvation on increasing the size of the vestigial wing. One remarkable result reported is that, if the treated individuals are allowed to breed, their offspring also have an increased wing size—even when only the father was treated. Eker has studied the effects of temperature on another mutant wing type and on the abnormal eyes associated with it. Harnly has continued his studies of the effects of temperature on the vestigial series.

Bodenstein has studied the bar-eyed type. Starvation, if complete, may shorten the larval life; if incomplete, it may lengthen it. Using this technique, and also the method of implanting eye discs between larvae of different ages, Bodenstein was able to show that the number of facets in a bar eye increases with the length of time the disc develops in a larva.

Epsteins has studied the effects of ultraviolet light in producing "phenocopies" in *Drosophila*.

NATURE OF CHROMOSOMES

Recent studies that may rather arbitrarily be classified as coming under the heading of cytology rather than of genetics, concern the nature of the chromosomes, from physical and chemical points

of view. The questions at issue are of importance to the geneticist, but all that can be done here is to call attention to the papers of Geitler; Painter; Mazia & Jaeger; Edlbacher; Caspersson & Schultz (1, 2); Metz; Gulick; Schultz, Caspersson & Aquilonius; and Wrinch.

HUMAN GENETICS

The first case of autosomal linkage in man has been recorded by Burks. Using an ingenious statistical method, she has shown a relation between a tooth deficiency and hair color. Though neither character is inherited simply and in a manner that is understood, it is clear that there is linkage of at least one of the genes concerned with each character with at least one of those concerned with the other. Linkage, between genes in man that show typical sex-linkage and no active genes in the Y, has been studied before, but in the few available examples crossing over has been rare. White now shows that there is at least 40 per cent recombination between green-blindness and night-blindness.

Papers on the inheritance of specific characters in man are too numerous to be discussed. One result of unusual interest may be mentioned. Lennox, Gibbs & Gibbs have studied the electroencephalographs of epileptics and of their relatives. It appears that there is a characteristic pattern present in all epileptics, and the evidence indicates that it is this pattern that is inherited—as a simple dominant character. It is present in about 6 per cent of a sample of people unrelated to epileptics; apparently something like one in twenty persons with this pattern develops epilepsy.

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DEVELOPMENTAL PHYSIOLOGY

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In making a selection from the rich production of new publications, emphasis has been placed in this review on work relating to problems of embryonic determination. Quite naturally, many of the papers deal with topics bordering the fields of biochemistry and genetics. Due to the European war, the most recent continental literature was not available for consideration.

Fertilization.—The last two years saw a sudden rebirth of interest in the chemical aspects of the fertilization process. The series of important publications opens with the report of Kuhn, Moewus & Jerchel (75) on carotenoids which bring about copulation between the male and female gametes of algae belonging to the genus, *Chlamydomonas*. The gametes secrete *cis*- and *trans*-crocetin-dimethylester, the *cis*- form prevailing in the female and the *trans*- form in the male. The substances serve by attracting mutually the gametes. Hartmann & Schartau (65), resuming work on the sea urchin, the classical object of the pioneer investigations by J. de Meyer and F. R. Lillie, were able to separate two principles from the egg cytoplasm; while the first one activates and attracts spermatozoa, the second one brings about their agglutination. Kuhn & Wallenfels (76) established the structure of the first principle, known for some time as echinochrome, $C_{12}H_{10}O_7$. Tyler (134) obtained no activation of *Strongylocentrotus* eggs with echinochrome. A number of vegetable and synthetic dyes closely related to echinochrome produce also the sperm activation and even the agglutination reaction. According to Tyler & Fox (136), the second principle or agglutinin (probably Lillie's fertilizin) is either a protein or very closely associated with protein, but Frank (47) supports earlier investigators who reported that it does not give the usual protein tests.

Meanwhile, interest has been focussed also on the male gametes (47, 122). Sperm extracts have been prepared and studied since Spallanzani. The following physiological effects are said to be produced: (a) inactivation of spermatozoan motility; (b) neutralization of the sperm-activating principle (echinochrome) of the egg; (c) dissolution of egg membranes; (d) neutralization of the sperm

agglutinin of the egg—anti-agglutinin of Tyler (135); (e) agglutination of jelly and cortex of eggs—egg agglutination (47); (f) partial activation of unfertilized eggs—not supported by Frank and Pincus (47, 103). These effects are probably produced by only two or three different substances. According to a recent short note by Hartmann *et al.* (64), the effects (a) and (b) may be obtained with a methanol extract of dried sperm, while the residue produces (c) and (d). Frank (47) finds the egg agglutinin in the protein residue; it resembles fertilizin in being nondialyzable, thermostable, and colloidal, but does not give the usual protein tests. On the contrary, Tyler (133) extracted lysins from mollusc sperm that appear to be true proteins. The Hartmann group proposes to designate the fertilization substances as gamones, distinguishing between andro- and gynogamones according to their origin. An interesting line of research is followed by Henle, Henle & Chambers (66), who describe agglutination reactions in bull sperm treated with antisera prepared in the rabbit.

Parthenogenesis and polyploidy.—Recent progress in the field of activation of the unfertilized egg is mainly related to vertebrates. Pincus (102, 104) has continued his work on the rabbit egg, using as stimulating agents hypotonic and hypertonic media, but also, with greatest success, exposure to low temperature (cooling at 6°C. for ten to thirty minutes). In one instance, birth of a normal living female that developed from an unfertilized cooled egg is reported. Breeding experiments with females obtained by parthenogenesis indicate that artificially activated eggs may be heterozygous. This leads to the conclusion that eggs become diploid by fusion of the sister chromosome sets of the second maturation division and that gene segregation can occur in the second meiotic division. First attempts at artificial stimulation of human eggs are reported by Reimann & Miller (114) and by Pincus & Saunders (103). In lower vertebrates, the double treatment of Loeb has been applied successfully to the eggs of lampreys (83). Most extensively used is the method of Bataillon, i. e., stimulation of the development of uterine frog eggs by pricking with a fine needle. Kawamura (71, 72) reports on very impressive materials (413 specimens) obtained in this way, and in which chromosome counts were made. Nearly one-half were haploid, one-fourth diploid, and one-fourth triploid; relatively few individuals had higher or mixed sets of chromosomes. Cleavage was delayed by one

step in the diploid group, indicating duplication of the haploid chromosome set while the first cell division was suppressed. Interestingly, no delay occurred in the cleavage of triploids. This leads to the assumption that three of the potential four nuclei resulting from meiosis unite into one fusion nucleus while at most one may be extruded in a single polar body. This interpretation receives some support from the observation by Parmenter (93) that eggs with two polar bodies furnish haploids and diploids, but not triploids. Of the three nuclear types the haploids have the lowest and the diploids the highest viability. In Kawamura's experiment, only twenty-three parthenogenetically produced larvae were able to transform into frogs, and of these twenty-two seem to be diploid and one triploid. The sex condition of these frogs is of some interest. The suspected triploid has sterile, rudimentary, testis-like gonads. Of the twenty-two diploids, eighteen have ovaries, and four have ovotestes or abnormal testes. There exists a close relation between bodily deformations and transformations of the gonads, which leads Kawamura to presume that all diploids are genetically of the female constitution, even those which phenotypically suffer a male transformation.

Triploidy, observed frequently in parthenogenetic eggs, may be produced also during normal fertilization. Perfecting a technique originally developed by Rostand (119), Fankhauser & Griffiths (42) obtained triploids in considerable numbers by exposing freshly fertilized eggs of newts to temperatures varying from 0.5° to 3°C. for sixteen to twenty-six hours. Most likely triploidy results from the union of the sperm nucleus with both daughter nuclei of the second meiotic division. Investigation of four metamorphosing triploids showed rudimentary ovaries in three and normal testes in one (41). This may indicate that in newts the female sex is normally heterozygous with respect to sex determining genes.

Teratogenesis due to nuclear conditions.—Poulson (107) studied the effect of X-chromosome deficiencies on the course of embryonic development in *Drosophila*. In the complete absence of X-chromosomes (Null X), abnormality becomes visible during the early cleavage stage (first hour). No blastoderm is formed. If half an X is present, either an incomplete blastoderm or one that fails to separate into germ layers represents the limit of development. An X with the relatively small deficiency, Notch-8, still gives very abnormal embryos, in which the nervous system fails to separate

from the rest of the ectoderm and hypertrophies, while the mesoderm fails to differentiate. Scute 8 is a very small deficiency which causes relatively minor defects that appear not before the late embryonic stages. In general, the defects are in proportion to the size of the chromosomal deficiency. Further investigations will have to decide whether discrete genes relate to definite events of embryogenesis, or whether the effects are linked to the quantity of chromatin deficiency. If the first alternative proves true, Poulson's observations would appear related to those by Dunn and collaborators (34, 51) who report on the inheritance of three alleles of taillessness: T , t^0 and t^1 . Homozygous individuals of each of these mutations die early: t^1t^1 before, t^0t^0 shortly after uterine implantation, and TT on the eleventh day of intrauterine life. The dominant T causes mainly a deficiency or complete absence of the notochord, while the recessive t mutations affect the mesoderm development.

A third type of important evidence for the role that chromosomes may play in teratogenesis comes from Baltzer's laboratory (4). By removing the nucleus from mature eggs of newts and fertilizing them with sperm of other species, so-called hybrid merogones were obtained, i. e., combinations of ovoplasm of one species and a sperm nucleus of another species, which show a remarkable vitality during early developmental stages. During the late blastula, the early gastrula, or the neurula stage, certain combinations pass through a critical period of nuclear degeneration. It is interesting, however, that all nuclei that escape destruction at this time later on prove capable of normal development. Evidently a condition of tolerance becomes established between the disharmonious components of this system. While the hybrid merogones, due to the extent of degeneration and other conditions of weakness, cannot be raised beyond early embryonic stages, transplants of fragments to normal hosts survive much longer. In two cases, Hadorn was able to carry such transplants even through metamorphosis. Epidermal characters in these instances were of the maternal type. This seems to suggest that the paternal nuclei have no influence on the development of hereditary characters in these hybrid merogones, although they help in the maintenance of the general life functions.

Mechanics of gastrulation.—Moore & Burt (84), in experiments on echinoderms, show that cutting away the embryo above the

endodermal plate in late blastulae and early gastrulae does not affect invagination. The factors responsible for invagination are contained within the endodermal plate. It is suggested that differential cohesion of the endodermal cells may be an important factor in producing embryonic folds and pockets.

In a recent paper, Child (23) reports on a reinvestigation of the effects of lithium on gastrulation of echinoderms, especially *Dendraster*. He concludes that exogastrulation results primarily from nonspecific differential inhibition (afflicting prospective ectoderm more than prospective endoderm) rather than from specific effects of lithium or other agents. Crowding in *Dendraster* and administration of Janus green in the starfish *Patiria* produce similar inhibitions and endodermization. This paper contains also an extensive review of Child's physiological gradient concept in its application to the problems of the origin of embryonic organization.

In spite of the application of new techniques such as color marking and explantation, there is no complete consensus of opinion reached yet as to the mode of endoderm formation in birds and reptiles. Peter (98) very definitely denies the participation of invagination movements, the whole endoderm forming by delamination from the epiblast of the blastodisc.

Pasteels' observations on the chick (94) are essentially identical with those of Peter. Their main difference seems to be one of terminology, Pasteels speaking of a "diffuse polyinvagination" of cells which in small groups lie scattered in the center as well as the marginal zones of the pellucid area. Jacobson (70) describes a difference in the origin of the yolk endoderm and the embryonic endoderm, the former supposedly forming by delamination in the area opaca, the latter by ingrowth of single cells from a circumscribed area, the "primitive plate." All recent investigators dispute the reality of endoderm formation by a turning inward of the posterior edge of the blastodisc—a mode described by earlier workers and still so represented in several textbooks.

Prospective fate, autonomous potency and competence of embryonic areas.—In the amphibian blastula, every district may be considered under three different viewpoints.

(a) What it differentiates into in the course of normal development is its prospective fate. The well-known maps by Vogt show the distribution of districts with respect to this prospective fate.

(b) Many districts can, however, under the influence of un-

usual inductor substances, produce a variety of different structures, as has been shown by the experiments of Hans Spemann and many others. Waddington (139) has proposed to include in the term competence (*Reaktionsfähigkeit*) all the possible reactions which a tissue may produce under any sort of stimulation.

(c) Finally, in recent experiments, Holtfreter (67, 68) studied the autonomous potencies of isolated fragments of early gastrulae which by culture in neutral media were protected from any outside induction. The results of his work are ably reviewed and discussed by Lehmann (77).

Considering that in normal development of amphibians, inductive processes play an important role, it seems only natural that the distribution of autonomous potencies does not coincide with that of the prospective fates. The region of the organizer, i. e., the dorsal lip of the blastopore of the early gastrula, is distinguished as much by its great variety of autonomous potencies as by its well-known inductive capacity. Relatively manifold potencies manifest themselves also in the prospective notochord and muscle segment regions, though with a decline in the ventral direction. This versatility appearing in the autonomous differentiation into tissues not normally developed from these areas indicates that the inductive substances are distributed in a crescent or nearly belt shaped area around or below the equator, with highest concentration in the upper lip of the blastopore. As a contrast, the areas of prospective endoderm (foregut, liver, esophagus, small intestine), heart, and kidney are very firmly determined and show a high degree of morphological and physiological self-differentiation. The fragments of intestine establish peristaltic movements and the primitive hearts developed from isolated fragments begin to pulsate at the normal time. On the other side, the prospective ectoderm has little inherent ability to differentiate. The only tissue type formed is that of a primitive epidermis. Medullary plate and neural tissue differentiate only after contact with inductive material.

It is of interest that in ascidians the whole blastula is in a condition of rigid determination resembling that of the vegetal parts of the amphibian blastula. In two series of experiments, Ubisch (137) shows that the major organ-forming areas are strictly determined as early as the two-cell stage. At the blastula stage, the organ-forming areas can be removed one by one. As a result,

embryos and larvae develop that are normal except for the lack of notochord, mesoderm, nervous system, epidermis or endoderm, according to the kind of prospective area that was eliminated. All parts are self-differentiating, and there is no evidence of induction nor of responsiveness to induction. With slight exaggeration, one may express this situation as: *Prospective Fate = Autonomous Potency = Competence*.

Hörstadius (69) has written a lucid review in English of his comprehensive studies on differentiation in echinoderm eggs and gastrulae. In echinoderms, as also in the chick, inductive processes play an important part in the determination of the embryonic primordia.

Much detailed work remains to be done to clarify the conditions prevailing in the bird blastodisc. The distribution of prospective primordia has been outlined by Pasteels (94), while Rawles, by the method of transplantation to the chorio-allantoic membranes, has tested the autonomous potencies of small pieces from all parts of the blastoderm of the head process stage. The fact that, according to Pasteels' studies with color marks, the upper part of the early primitive streak should be lined with mesoderm, while Rawles (110) and Eastlick (38) find here the precursors of pigment cells, suggests similar differences in the development of whole embryos and small fragments as they exist in amphibians. Twisselmann (131) obtains new evidence for extensive regulative potencies in the lower part of the blastodisc of ten hours' incubation, while Morita's experiments (87, 88) had shown that formation of several accessory embryos may be induced by implanted dead organizers. As a whole, determination in birds evidently follows more nearly the amphibian than the ascidian type.

The regulative potencies of orthopteran embryonic discs have been studied by Krause (73). Pricking the eggs of tachycines with fine glass needles causes the formation of small extraovates which carry with them parts of the discs. Before invagination and gastrulation, the restitutions are considerable. Whole embryos form even after elimination of an entire lateral half of the disc. Bilateral-ity becomes fixed, however, during gastrulation and, following this event, all morphologically distinct districts become unreplaceable. The differentiation center is located in the prothorax. From here, physiological and morphological differentiation proceeds in all directions. Only the prostomial region manifests some capacity for

self-differentiation in the independent development of eyes and ganglia.

Fractionation of the organizer.—As early as ten years ago, Spemann made a definite statement concerning regional differences in the amphibian organizer. Mangold and, later, Hall (54) have further elaborated this concept, distinguishing between head and trunk organizers. Recently, Eakin (36) found corresponding inductive differences in the roof of the archenteron of the midgastrula of a teleost (trout). In tests with indifferent extraembryonic ectoderm, the most anterior portion of the archenteron showed little or no power of induction. The middle portion induced mainly brain and auditory vesicles (head organizer) and the hind portion "neural structures, spinal in character" (trunk organizer). Of particular importance appear to be the findings of Chuang (24) who reports that in tests on ventral ectoderm of *Triton* gastrulae, mouse liver acts like head organizer while *Triton* liver acts like trunk organizer. The prospective epidermis is not only induced to form neural but also mesodermal structures. Significant seems to be the further statement that through boiling, both tissues lose first the capacity to induce mesodermal structures, then central nervous system, and lastly nose and eyes. It is concluded that there exist at least two inductive principles which differ in their resistance to boiling.

Chemical nature of the organizer.—The actual crisis stage which this field of research has reached is most competently characterized by Needham (89) as follows:

It may be said that although the progress made in the last ten years in these fields has been very great, we can nevertheless see now that owing to the special difficulties of the subject, especially perhaps the presence of evocator in ventral ectoderm, it may be more like fifty years before we can expect to have certain knowledge concerning the chemical nature of the naturally-occurring substances involved in embryonic induction. Like so many other biological problems, this has turned out to be more complex than the first explorers thought. But that is not a pessimistic conclusion.

Waddington (139) has collected in a fascinating small volume of 160 pages the many contributions to this subject, and Needham has prepared for publication a more encyclopedic text of about 1000 pages ("*Biochemistry and Morphogenesis*"). Difficulties arise from two major sources. First, induction effects are obtained from a multitude of chemicals as different in nature as proteins (6), hydro-

carbons (121), and inorganic substances like silica or calcium carbonate (91). Second, all parts of the late amphibian blastula and early gastrula, with the probable exception of the prospective endoderm, contain inductor substance in inactive, probably conjugated form, which may be set free by cytolyzing agents. Since ventral ectoderm of early gastrulae is mainly used as an indicator, the difficulties of the situation are only too obvious. In comparative tests, it has been found, however, that some estrogenic and carcinogenic hydrocarbons are by far more active than any of the other substances, producing inductive effects even in extreme dilutions. An observation by Waddington seems worthy of more consideration, namely that inductive substances have to be applied to restricted areas. It would seem, therefore, that gradients of concentration have to be established in the tissues.

In a short note, Barth (7) reports on induction without organizer substance. If isolated pieces of presumptive epidermis or presumptive medullary plate were folded in the direction of the main axis of the blastula, neural tubes formed; if folded transversely, neural tubes formed but rarely. While decisive gradients evidently would have to rest on concentrations of catalysts, they may reveal themselves by secondary differences in metabolism. Almost simultaneously, several laboratories have contributed to give a picture of the particular metabolic conditions in the organizer tissue, i. e., the upper blastopore lip of the gastrula. According to Piepho (101), Barth (8), and Brachet (16), the dorsal side of most blastulae and early gastrulae has a higher respiratory rate than the ventral side. However, the inductor region does not respire at a higher rate than that of the dorsal ectoderm (prospective medullary plate). Boell & Needham (14) observe that the blastopore lip is distinguished by higher anaerobic glycolysis than other parts of the gastrula. The same investigators and Brachet (16, 17) find also indications of a specific protein metabolism in the same district (increase in protoplasm at the expense of yolk).

The significant accumulation of sulphydryl compounds will be mentioned in the next paragraph. Caution is advisable in the evaluation of these findings. Whether they are specifically related to the induction phenomenon may be doubted in view of the fact that in the amphibian embryo as a whole, metabolism changes very rapidly (144). Some of the differences merely indicate that within one embryo certain parts run ahead of others with their

developmental changes. Furthermore, as Brachet (16) emphasizes, the secondary importance of oxidation rates is brought out by the fact that induction may proceed under anaerobic conditions or in explants treated with cyanide. Similar reservations are made by Needham & Needham (90) in regard to Lindahl's attempt (80) at an interpretation of determination gradients in the sea urchin blastula in terms of metabolic gradients (animal and vegetal types).

Bilaterality and polarity.—The oldest topic of experimental embryology, determination of the plane of bilateral symmetry in the frog egg, has been reinvestigated by French and Belgian workers. Until recently, Pasteels, like many others, had assumed that this determination was made already in the unfertilized amphibian egg. Under the influence of reports by Ancel & Vintemberger (2) however, he had to admit the possibility that in some instances the decision depends on the orientation of the egg before the time of its liberation from the vitelline membrane. If before this time the main axis of the egg stands obliquely, the top side of the egg becomes the dorsal side. The important role which gravitational forces can assume in dislocating the primary axes of the egg of one or two cells becomes emphasized again by a repetition and extension of Schultze's classical inversion experiments (95, 96, 97). The very interesting microchemical work of Brachet (16, 17) suggests that the determination of the dorsal side may depend on the distribution of protein sulphydryl groups which originally are contained in the nuclear sap of the germinal vesicle. Waddington (138) lends some support to the notion that the nuclear sap contains organizer substance. He finds that germinal vesicles of immature *Triton* eggs give more and larger inductions than comparable pieces of cytoplasmic material. On the other hand, Brachet's work on sulphydryl compounds has found criticism by Giolitti (50) who questions the value of the nitroprusside method and emphasizes the failure to obtain localized color reactions in the eggs of the lamprey.

Dalcq (25), in experiments with fertilized fragments of *Asci-diella* eggs, established the presence of a dorsoventral field in the cortical layer of the unfertilized egg. Its maximal concentration in a region near the equator designates the dorsal side and thereby the second (dorsoventral) main axis of the egg. Ries (115), making chemical tests for oxidase, peroxidase, and glutathione in ascidian

eggs, finds that the reactions are not specifically localized in the ovocytes. But, even after fertilization, the peroxidase and oxidase reactions become centered in the yellow myogenic crescent that marks the ventral side of the egg. In centrifuged and in operated embryos, the reactions seem always to be linked with the myoplasm, but not with the pigment (137). These tests support the conclusion already reached by Conklin that the yellow pigment is not a muscle-inducing substance.

New reports by Streett (127) and Pasteels (97) show again that a bilateral structure may be found in unsegmented eggs, though the problem of its origin remains undecided.

An attempt to approach the problem with new methods was made by Harrison, Astbury & Rudall (63). Starting from the observation that certain embryonic tissues are first isotropic and later become polarized, an attempt was made to detect in differentiating tissues by means of x-ray diffraction photographs, some structural elements indicative of molecular orientation. The negative results obtained so far are not decisive with respect to the "molecular orientation theory of tissue polarization."

Although an analysis of all inductive processes following the neurala stage is not attempted, reference must be made to the important publications by Hall (55) and Swett (128) on the establishment of polarity in the ears and limbs of salamanders, by Stone *et al.* and Zwilling on development of sense organs (123, 125, 126, 152), and by Detwiler (30, 31) and Hamburger (56, 57, 58) on neural and neuromuscular relationships.

Origin and morphogenetic potencies of regeneration blastemas.—

The problem of the extent to which adult tissues recover an embryonic condition of indeterminacy, and competence to respond to inductive agents, has received renewed attention, thanks to the work of Schotté (120) on the tail and limb regeneration blastemas of amphibians. Schotté reports that with the proper inductive stimuli, the formation of lens, ear vesicle, olfactory pit, and mouth cavity out of regeneration blastemas can be elicited. Stone & Sapir (124) and Emerson (39) have not been able to corroborate Schotté fully. As Weiss (140) points out, one can object in most instances that the two components, inductive and responsive tissues, are not sufficiently different to decide later the true origin of the regenerated structures. Tagging with vital stains or use of nonliving inductor substances may help to clarify the situation.

Morphogenetic substances from the brain and the corpora allata of insects.—Kopeć, as early as 1922, had suggested that the brain of lepidopterans produces a pupation hormone. In recent years, a large number of investigators have followed this lead, and after some initial uncertainty as to the specific role of the corpora allata, the assumption of Kopeć now stands generally accepted (15, 74, 105). It appears that the corpora allata of grasshoppers have nothing to do with the molting process (99, 100). In the hemipteran *Rhodnius*, they merely seem to produce the inhibitory "nymphal" hormone, which prevents the development of some adult characters, possibly by the production of a coenzyme. Wigglesworth (141) finds that it is the "dorsal region of the protocerebrum" which produces the molting hormone. According to Pfeiffer (99), the corpora allata control the final growth of the eggs and the secretory functions of the oviducts. The ring gland of dipterans contains not only the corpora allata but also the esophageal ganglia. Bodenstein (11) has published interesting studies on the induction of metamorphosis and pupal differentiation in *Drosophila*, under various operative and experimental conditions.

Time relationships of inductive processes.—Enzymes, hormones, and other activating substances depend in their manifestations on the presence of responsive tissues. An interesting situation has been shown to exist in regard to the sex ducts in salamanders (*Ambystoma*). The first phase of their normal development proceeds by self-differentiation, which in this connection means merely without control by hormones of the sex glands. In all hatching larvae (one and one-half months) of *A. opacum*, the future male ducts extend from the pronephros to the cloaca, and they persist in full length until the approach of metamorphosis, when the parts above the mesonephros degenerate (five months). The oviducts begin development only during the third or fourth month, growing down from the pronephric bodies, and reaching the cloaca at or shortly after metamorphosis. The work by Rodgers & Risley (117) and Foote (44, 45) shows that the male ducts begin to respond to injected sex hormones at the age of four to five months, while appreciable amounts of hormone are produced by the testis beginning with the seventh month. Responsiveness to androgenic hormones is totally absent in the ducts during the first phase, but becomes established at least two months before the appearance of activators in the normal course of development. The change is

not linked with any obvious morphological events in the cells. The situation is somewhat different in the case of the oviducts. It was found that the oviducts respond even more extensively to testosterone than to estrogenic hormones, if given in doses of equal weight, and that the oviduct tissue is responsive from its first appearance in the third month. As in the male ducts, responsiveness is ahead of the actual appearance of normal activating hormones by about two months, though there is no unresponsive phase in oviduct development. A further point of interest appears in the fact that physiological stimulation of oviduct tissue destroys its capacity of embryonic growth. As soon as hormone stimulation is initiated, the downward growth of the oviduct rudiments stops while their diameters enlarge (functional growth) due to the development of the mucosal layers.

Somewhat similar conditions seem to prevail in the chick where the basic morphological differentiations are also independent of gonadal control. Fugo's experiments (48) on hypophysectomy on the second day of incubation suggest that self-differentiation in the chick goes even beyond the stage reached in the salamander. In the absence of gonadal hormones, the initial development of paired oviducts is followed in the male by the complete reduction of both oviducts and in the female by the characteristic regression of the right one only. This feature of sexual differentiation appears, therefore, as under a more direct genetical control. It is the more remarkable that in both sexes of the chick, experimental hormone stimulation can again bring about arrest and fixation of primitive embryonic conditions, resulting sometimes in persistence of paired oviducts in both sexes. The literature on this subject is fully reviewed by Willier (142). As in amphibians, the oviducts of birds are stimulated by androgens as well as by gynogenic hormones (149).

It is not surprising that conditions of responsiveness become changed in mammals, where the developing embryo is exposed to the hormones produced by the pregnant mother. In the opossum, with a gestation period of only thirteen days, the responses of the ducts follow still the same lines as in amphibians and birds, i. e., the ducts become responsive at an early stage. The oviducts also become strongly stimulated by administration of testosterone as well as of estrogens (20, 21, 85).

The situation changes considerably in rodents and obviously

also in man. The very incomplete experimental data from many laboratories have recently been compiled by Greene *et al.* (52). The first general impression is that of a very low responsiveness of the ducts of fetuses. Even with extreme doses of androgenic hormones, the males show no stimulation. However, under these conditions, a considerable development and persistence of male ducts and their glands are produced in the females. In the reverse experiment, no effects are produced with estrogens except with excessively high dosages. If these high doses are administered, deviations appear in both sexes which Greene *et al.* appraise as masculinizations in the female and feminizations in the male. Essentially, the effects consist in a retention of mullerian duct derivatives in the males and of wolffian duct derivatives in the females. To the reviewer, it appears that specific stimulation effects are practically absent and that the observed abnormalities may be considered as unspecific inhibitions leading to persistence of embryonic conditions. The fact that responsiveness to gynogenic hormones is more nearly completely lost than that to androgens must be considered as an adaptation to the prevalence of the former in the embryonic environment.

Changes in responsiveness as well as changes in time of release of appreciable amounts of hormones (and other diffusable activators) hold an important place in evolution. In salamanders, there occurs no sexual differentiation in the duct systems in prepuberal stages. In birds and mammals, however, an extensive dimorphism of secondary sex characters develops by self-differentiation long before the time of release of sex hormones in physiological threshold quantities. The importance of genetical conditions for the development of contrary sex characters is emphasized by work of Mahoney (81) on self differentiating prostates in female rats.

That certain hormones may appear before the effector system has acquired the capacity of response is indicated by some recent experiments with chick embryos. In normal development, the thyroids give no indication of stimulation by the hypophyseal thyrotrophic hormone before the eleventh day of incubation, while transplanted thyroids from hypophysectomized donors react from one to two days earlier (81a).

In grasshoppers, Bodine & Allen (12, 13) find considerable amounts of tyrosinase (protyrosinase and activator) in eggs long before the corresponding substrate and before melanin production

starts. Similarly the so-called v^+ eye-color hormone of *Drosophila* (9), which seems to be identical with the a^+ hormone of the moth *Ephestia* (106), is present long before the effector systems are ready for reactions. In fact, in the a^+ *Ephestia*, it is present throughout life and may be handed down by a heterozygous mother through the cell body of an a^- egg to her offspring. This represents a form of maternal inheritance which has been described by Plagge under the term of predetermination. The v^+ hormone, which is known to be produced in various organs and tissues in a variety of insects, can be synthesized also by certain bacteria (129).

Sex determination.—Efforts in this field center around the problem of a possible role of hormones in the determination of primary sex differentiation. For comprehensive reviews of the literature up to 1938, see Willier (142) and Witschi (146). In mammals, injection of large amounts of hormones into pregnant mothers has not changed the sex type (28, 52, 111, 130), nor have these experiments contributed to clarify the problems offered by the cattle freemartins (85, 146).

In birds, apparently nothing essential has been added to the situation as described in Willier's review. Gynogenic and, to a restricted degree, androgenic hormones (androsterone) produce a partial sex reversal in males. The cortex of the left gonad persists and differentiates in the ovarian sense while the medulla, especially the left one, is temporarily suppressed. It remains undecided whether in rare extreme cases, permanent complete sex inversions may be produced by the estrogens. Rather perplexing are the effects of androsterone; they represent mixtures of masculinization and feminization, probably in both genetical sexes. Conditions in reptiles (29, 116) seem to be similar to those in birds.

Most extensive reactions are produced in amphibians. Puckett (108) and Hanaoka (60) report that races of frogs and salamanders of the undifferentiated (partially hermaphroditic) type differentiate early into equal numbers of males and females after the application of hypophyseal substances. With respect to their reactions toward sex hormones, there seems to be a striking difference between salamanders and frogs. In the latter, androgens cause genetical female embryos to develop like normal males (literature in 146, 147) and the ovaries of female larvae to transform into testes (46). Gallien (49) and Puckett (109) report that in undifferentiated races, the juvenile sex ratio of 100 females:0

males is maintained beyond metamorphosis following administration of gynogens—a result the importance of which cannot be fully valued unless such offspring are raised to near or full maturity. In differentiated races, the gynogens in no way affect the normal testicular development (46). However, they prove most effective in salamanders. If treatments start early enough, i. e., before the disappearance of the embryonic cortical rudiments, the genetic males respond by developing ovaries or ovotestes, thus bringing the sex distribution more or less close to the extreme of 100 per cent females (1, 19, 44, 61). On the other hand, androgens interfere little with normal sex development, and if so, in a paradoxical, feminizing sense (147). Summing up, it appears that salamanders, reptiles, and birds react mainly to female sex hormones and by changes appearing as feminizations; frogs and mammals (the latter in a more limited extent) respond more to male sex hormones by showing effects of masculinization.

The results obtained with sex hormones in salamanders are of a particular interest, for they furnish unequivocal proof of the nonidentity of sex hormones and embryonic inductors of sex differentiation [corticin and medullarin of Witschi (146, 147)]. This conclusion is derived especially from a comparison of the effects of hormone treatments with those from parabiosis.

At the present time, it is still impossible to interpret the conflicting notes and incomplete reports on effects produced by sex hormones in fish (3, 10, 18, 40, 53, 92, 113, 146).

Here should be mentioned also the important work by Moewus and his collaborators (75, 82) on the role of carotenoid derivatives in the sex determination of algae (see also p. 57).

Determination of color pattern.—Much evidence from recent investigations (35, 38, 62, 110, 132) points to a common origin of all melanophores of amphibians and birds (possibly of all vertebrates) from cells of the embryonic neural crest. In amphibians, DuShane (35), Twitty & Bodenstein (132), and Rosin (118) have transplanted embryonal chromatophores to hosts of different race, species, or genus. In the foreign environment, the chromatophores display a considerable faculty of autonomous cellular development and pattern formation. However, certain restrictions are set by host tissues, as for instance, by the ventral surface of *Triturus* or by nearly the whole surface of white axolotls. It seems, therefore, that the small patterns, such as shape and size of spots, are mainly

determined by factors within the chromatophores, but the large pattern by the tissues, ectodermal and mesodermal, with which they come in contact.

Pigment cells of the bird give evidence of remarkable capacities for autonomous growth, differentiation, and migration, if transplanted into host tissues of different breed (or species) or placed in culture *in vitro*. Neural crest material of the first or second day of incubation grown in a clot of adult fowl plasma plus embryonic extract shows actual differentiation of pigment at the end of the second day. This is much earlier than *in situ*. Furthermore, explants from White Leghorns also give pigment-producing melanophores (32). According to Hamilton (59), all white breeds contain black pigment-producing melanophores which die sooner than corresponding melanophores from black breeds. It is suggested that feathers are white because most of the melanophores die before they can deposit much pigment. Red pigment-producing melanophores occur only in red-feathered birds. *In vitro*, they require the addition of sex hormones to the culture medium in order to produce pigment. Pigment cells from mature cultures have successfully been grafted back into early embryos. Here they maintain the character of adult melanophores, multiplying but slowly and always retaining their pigment (33).

The direction of migration of the embryonic melanophores has been demonstrated by grafting. Limb buds from twenty-four to thirty somite embryos of colored breed transplanted into the coelom of any kind of embryo will later develop pigmented feathers if the transplant included tissues up to the neural tube. If, on the contrary, it was severed at a lower level (leaving out all melanoblasts!), the limbs develop only white feathers (37). Danforth, as early as twelve years ago, observed in skin transplants between Barred Plymouth Rock and nonbarred breeds some mosaic feathers belonging essentially to the nonbarred form, but containing melanophores of the Barred Rock. From the fact that these melanophores produced the typical bar pattern, he concluded that the pigmentoblasts are in direct control of pattern development (27). This fascinating problem has now received a very detailed study by Willier & Rawles (143). Experimenting with a variety of breeds of fowl and transplanting also embryonic melanophores from the robin to the chick, they can show that "the melanophore is a breed-specific cell whose action in a foreign feather germ is in

accord with its genotypic composition." However, certain details of pattern of distribution of pigment granules are under control of the host tissues. A final analysis of the problems of pattern production will greatly be helped by Lillie's detailed studies (78, 79) in the physiology of feather development. The role of enzymes and of hormones (59, 148, 150, 151) in the process of pigment production has also obtained renewed attention.

Pigment cell migration in mouse epidermis (112), the mechanism of the low temperature effect on pigments in Russian rabbits (26), and the importance of vitamins in the prevention of greying of the hair (86) are subjects of recent investigations in the field of mammalian pigmentation.

Physiology of the fetus.—Comprehensive reviews have been published by Windle (145) and by Barcroft & Barron (5), the latter on the movements in the mammalian fetus.

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GROWTH

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The postnatal increase in the size of the soma and of its components is the aspect of growth with which this review of the recent literature is concerned. The investigations that could be included under the heading of growth studies are so numerous and so heterogeneous in kind that it is necessary to restrict¹ the field of survey and to select according to some definite viewpoint the reports to be discussed. The selection of the reports examined here has been influenced by the concepts that underlie the objectives of human biological studies in general and those regarding public health in particular. This is to say that, while this review attempts to bring together the results of most of the recent important investigations on other animals as well as on man, attention is mainly focussed on observations concerning man and on findings on the lower animals that might clarify the manifestations in man of the phenomenon under discussion.

CHARACTERISTICS OF SOMATIC GROWTH

(a) *Stature and weight.*—During the period covered by this review studies on the mathematical formulation of growth have been very few and these limited to a reiteration of well-known conclusions. In addition, Raiford has examined stature and weight data on infants from two weeks to one year of age and fitted the mean dimensions with a second order parabola: $\text{age} = a_0 \div a_1x \div a_2x^2$. In this, x denotes either stature or weight. By a simple transformation the equation can be written to express stature or weight in terms of age. Obviously, the utility of such a curve is limited only to a short age range since an inflection in the growth curve is expected as observations both on man and other animals have repeatedly shown. Recently, Ittner & Hughes have presented data on young growing pigs to show just such a trend. At about 170 days

¹ The war has imposed further restrictions since it has been practically impossible in recent months to obtain many foreign journals.

of age the slope of the curve begins its reduction. These authors have not, however, described their observations in terms of a mathematical functional relationship.

The problem of arriving at some satisfactory "standards" of stature and weight in children is still largely unsolved and probably will be so long as the many factors besides nutrition that affect somatic growth are ignored by those who attempt to construct such standards. Metheny has examined the annual variation in the same child of McCloy's index of "normal weight for body build" and finds that it may change considerably from year to year. Metheny's study had for its purpose to determine whether the measurements for such an index need to be taken every year on a child in order to determine its "normal" weight for the period. In view of the findings she concludes that it is necessary; but these findings also reflect, it would seem, on the validity of the index. Another index that is supposed to express the "normal" physique of children is described by Tuxford. His index is based on the weight-stature ratio which he corrects for age so that "normal" has a value of unity. This correction factor is derived from the postulate that consecutive terms of a geometrical series of weights, divided by the corresponding terms of an arithmetical progression of statures, form a series of weight-stature ratios which are in harmonical progression. In the particular sample dealt with (English children) the correction for the weight-stature ratio using

metric measures is for boys equal to $\frac{(381 - \text{age in months})}{54}$ and for the girls equal to $\frac{(354 - \text{age in months})}{48}$. As have all such stand-

ardization procedures, this has a number of practical and theoretical weaknesses due to the method and variables employed.

Further clarification of the pattern of growth is provided by the latest of a series of monographs by Shuttleworth (1) in which he discusses the growth pattern in stature and weight as well as sitting height, leg length, iliac diameter, chest breadth and depth, and head width and length in terms of the age of the maximum increment of growth of stature. Shuttleworth's study, which is based on seriatim measurements taken on subjects from six to nineteen years of age, cannot be discussed in detail here, but among the significant findings one notes first of all that in girls the age of men-

arche is apparently highly correlated with age at maximum growth increment. Secondly, the children who demonstrate a maximum increment at an earlier age are more precocious in attaining final size. Thirdly, mature stature can be predicted with greater accuracy from the measures of childhood than those of adolescence. This last finding agrees with the conclusions reached by Bunak, who favors the theory that variations of final size are not related to duration of pubertal period or the rate of growth during this period. Summarizing published and unpublished data on tall groups of children, Bunak shows that as groups they may have characteristics of precocious, medium, or tardy development. As a means of classifying, relative to stature and weight, populations or groups in terms of the general level of growth intensity and the relative growth in different periods, the author proposes two indices. The first is given by the ratio of stature (or weight) at seven years of age to that at sixteen; the second by the ratio of the dimension at sixteen to that at twenty years of age. There is logic in this index which certainly deserves to be explored further.

Meredith's (1) observations on Massachusetts children of North European and Italian extraction are pertinent and in general agree with the above results. In particular, he notes that the annual increment of the two groups compared are practically the same although the final results are, as is known, different. The effects of retarded growth on final size are apparently different when the retardation is induced by lack of sufficient nutrition. Thus the experiments of McCay *et al.* show that rats maintained for periods on a diet that did not allow a weight increase never attained mature size. Incidentally, their findings demonstrate that, although weight was experimentally held constant, the growth of bones continued, although slowly.

A careful study of the stature, weight, and sexual maturation of obese children by Bruch is very interesting. The pattern of growth of these children is similar to other children although at a higher level. An attempt by Mullen to employ a variance analysis technique to determine the major conditioning factors in growth seems to indicate that it is not the most adequate tool for the purpose, at least as used.

The variability in stature and weight from birth to five years of age has been the object of an investigation by Peatman & Higgons, who bring out for this particular age period, as has already

been found for older children, that absolute variability increases with age, while relative variability decreases. The main point of this study is that the observations were made on children having optimal pediatric care. A very interesting series of observations on daily variations in the weight of mice is reported by Kopeć. He notes that in mature female mice the weight-growth curve proceeds in short cycles or waves, the first of which appears at maturity. No such characteristic is found in males, and in the females the oscillations become less in amplitude with the age of the animal. The probable significance of these facts will be discussed later in connection with Adamska's paper.

Individual growth curves for a number of somatic dimensions obtained on thirty-four infants from birth to one year of age measured at irregular intervals are discussed by Davenport (1). For some individuals and measurements the data may be fitted by the well-known growth formula but for others this is not the case and the author outlines various hypotheses that might explain the results. Individual serial data on stature and weight are also reported by Stuart and by Gray & Faber. The latter give the stature and weight curves obtained on two girls measured from birth to seventeen years of age. An interesting observation is that, although the girls belong to the higher socioeconomic classes, their weight curves show periods of marked oscillation apparently not accounted for by external conditions.

(b) *Other somatic dimensions.*—In the reports by Shuttleworth and by Davenport cited above, the growth of other somatic dimensions have been described with conclusions that do not differ from those regarding stature and weight. The body segments studied by Shuttleworth have been mentioned while those measured by Davenport in his observations on infants comprise practically every known dimension in the anthropometric repertoire. Davenport also gives statistics on the "errors" involved in the process of measuring.

Meredith (2) reports data on the main components of stature: head and neck length, leg length and trunk length, of children six to seventeen years of age. The last measurement was taken in the manner of the sitting height except that the height was measured at the suprasternale instead of at the vertex. Head and neck length were obtained by subtracting this trunk length from sitting height, while the leg length was obtained by subtracting sitting height

from stature. In view of the technique employed, Meredith's results are not strictly comparable to those already described in the literature. In any event, the author's data show that the head and neck length in proportion to stature decreases with age from six to seventeen years of age. The proportion that leg length is of stature increases from six to twelve years of age and decreases thereafter, while the reverse picture is presented by the proportion of trunk length to stature. The three dimensions mentioned demonstrate fairly high correlations among themselves.

Studies on the changes in size and shape of the head with age have been reported by Goldstein (1) and by Davenport (2). The former presents seriatim data on head length and width, and cephalic index of children six to seventeen years of age. One of his significant findings is that the cephalic index of over 30 per cent of the children changes from one index category to another, the most common change being from brachycephaly to mesocephaly. In addition, the author finds that, as for other somatic dimensions, girls with earlier menarche also have a more precocious development in head length and width. However, the final head size is practically the same in girls with early and late menarche. Davenport, in the paper mentioned, discusses briefly the age curve of the ratio of head height to head length. It is apparently a very complicated curve, especially in early childhood; the author believes that the absence of a smooth continuous trend is probably due to variations in posturing or similar mechanical factors.

Davenport's (3) publication on the development of the outer nose represents a comprehensive and thorough treatise on the subject. Based on cross-section as well as longitudinal measurements, it concerns five absolute dimensions and eight ratios. Nasal height increases in a manner parallel to stature showing also a growth spurt at puberty. He notes that in identical twins the growth curves are practically identical and in brothers they are parallel. Davenport's study contains an excellent discussion of the ontogeny as well as phylogeny of the nose. Supplementing in a way Davenport's monograph is a paper by Goldstein (2) on the vaulting of the nasal bridge. He finds that between three and seventeen years of age the nasal bridge diminishes in width at all levels from the apex. After about seventeen years of age the contraction of nasal bridge width stops and apparently a slow increase follows. The reduction in the width of bridge is supposed to be associated with

increase in the height of the bridge vault and it is on this basis that the author discusses his observations.

The weight of the eyeball has been studied by Todd and associates and they arrive at growth curves in terms of skeletal as well as chronological age. The main finding is that during the first year of life considerable growth takes place and by three years of age the weight of the eyeball almost reaches its adult level. Aside from its theoretical significance, this finding would seem to have a practical one relative to the problem of the development of eye defects in children.

Francis (1) gives data on the growth of the tibia as measured by roentgenographic and by anthropometric methods. The statistics have not been sufficiently elaborated, but it would seem that the rate of growth of the tibia diminishes slowly from two to twelve years of age and markedly thereafter for the girls, while for the boys the marked decrease in growth rate does not take place until after the fourteenth year. An observation of practical importance is that the roentgenographic method aside from being more expensive is not superior to the anthropometric technique in obtaining this measure. A similar conclusion is arrived at by Maresh & Deming, who, however, point out that the serial growth curves based on roentgenographic measurements showed less variations from calculated trend lines. Maresh & Deming's study concerns the growth of the long bones in infants from two weeks to twenty-four weeks of age. Their findings, which are discussed in detail, lead to the conclusion that during the first six months of life the growth of the long bones proceeds at a uniform rate. Employing data limited to anthropoids, Lumer discusses the problem of whether the power function $y = bx^a$ satisfies the data on the relative growth of limb bones. He finds that the formula expresses adequately the observations and discusses the evolutionary meaning of this fact.

The practical difficulties inherent in determining surface area have probably been one of the main reasons that not much has been done to utilize this measurement. Recently Weinbach (1) has developed an ingenious method of arriving at a contour map and surface area of the human body by employing stature, weight, and, in addition, certain measurements taken from photographs. From this, Weinbach (2) was led to a very simple formula which gives surface area as the product of stature by twice the right thigh

girth. He finds that the results correlate very highly with those obtained by other methods and outlines a curve of the growth of surface area. With regard to this dimension, Wilmer notes briefly the age changes in the proportion of skin to tela subcutanea per square centimeter of surface, finding that a tremendous change takes place during the fetal stage but practically none after birth.

One important aspect of somatic development which to date has only been slightly and inadequately considered is that regarding the correlations between somatic dimensions and between bodily organs. Some information on the relationship between stature, weight, and chest girth is given by Fujii on Japanese children, and the results confirm those already known. Comeau & White study heart size in identical twins and compare the differences with those of pairs of unrelated individuals having the same stature and weight. The authors reach the important conclusion that heart size in normal individuals is dependent principally on body build. The sample considered by the authors is small so that it would be necessary to investigate this question more extensively, although the conclusions agree in general with those from other studies on body build. Lung weight is proportional to body weight in the growing rat, Cohn notes, and when some lung tissue is removed there follows a period of more rapid growth of the lung. As a result, the lung weight becomes again proportionate to body weight. The interval of time for the restitution of lung weight is less in younger animals. The phenomenon, as the author shows, results from the mechanical stimulus related to the size of the thoracic cage.

(c) *Skeletal maturation.*—As a result of Todd's fundamental work, stimulus has been given to the study of the pattern of ossification in order to measure "anatomic" age and as another time scale in growth. With reference to Todd's atlas, Bayer & Newell point out, on the basis of seriatim observations on ten children, that variations may occur in the sequential order of appearance of the ossification centers. They have limited themselves, however, to a study of the skeletal development of the hand and knee only. Additional and more detailed data on the time and order of appearance of ossification centers from birth to five years are given by Francis & Werle and from six to fifteen years by Francis (2). These data concern the first 20 per cent, or more precocious, children examined. Since this sample of children also belongs to the higher

socioeconomic classes and has been reared in better than average home conditions, the standards given may be considered those for optimum environmental conditions. Graphic standards based on children seen in the Harvard Center are published by Vogt & Vickers. These concern the ossification centers of the hand and foot from birth to $6\frac{1}{2}$ years. The standards are arranged to illustrate the skeletal maturation of advanced, average, and slow children. Sontag *et al.* seek to simplify considerably the problem of evaluating ossification, at least with regard to young children. They propose to limit the appraisal of development to a count of the centers appearing in all the joints of the left upper and lower extremities. The average frequency of the centers according to age, up to five years, has the usual characteristics of a growth curve. The possibilities of such a technique certainly merit some attention.

ELEMENTS OF VARIATION IN GROWTH

(a) *Secular changes.*—The most striking phenomenon relative to human growth in the last half century is the apparent progressive increase in stature and weight that many investigations have shown in children especially. Additional information on this point is given by Gould, who examines data collected since 1909 on female students of Newcomb College. The students of earlier years were shorter on entrance, grew more in stature, and showed more seasonal fluctuation in growth after entering college than the students of equal ages of more recent years. This would seem to indicate then that the recent generations have a more precocious development. Jacob studies data on girls who attended St. Paul's School (England) from 1907. The usual trend toward an increase in recent years is observed for all ages and in both stature and weight. Since the school has always been attended by girls of the higher social classes, the author doubts whether the increase is due to improvement of diet.

Increase in the size of the head and face is discussed by Boas, who compares a sample of American-born Hebrew children measured in 1906 with a sample measured in 1936. The head length and width have increased in absolute dimension but not relative to stature. Face width has increased in absolute and relative size.

The view of the majority of students of the subject is that dietary improvement and other social factors are responsible for

the increased size. Other explanations have also been advanced. Mills & Chenoweth, for example, consider the phenomenon as a manifestation of a trend toward precocious maturation. This, they believe, is in turn the result of subnormal temperature levels in the latter part of the nineteenth century. A marked rise in temperature level has been noted since 1920 and therefore the authors expect both a retardation of maturation and a diminution of growth to reverse the trend observed so far. In fact, they present data to demonstrate such a reversal but the statistics are not very conclusive. However, that other factors besides dietary ones may have affected the growth phenomenon observed should not be overlooked. Shuttleworth's (2) report on annual increments in stature and weight reveal that significant variations occur from year to year in the same children. Based on *seriatim* measurements taken annually from 1922 to 1935, Shuttleworth's analysis brings out the fact that for stature between 1923 and 1924 the average increment was above normal, in the following three years it fell below normal and oscillated thereafter. Similarly, the increments in weight oscillated from one year to another. No explanation is given.

(b) *General and unspecified factors.*—Stimulation or inhibition of somatic growth is brought about by the action of several mechanisms. In terms of the limited knowledge about them one can distinguish specific ones such as certain hormones and vitamins—factors that are relatively sharply individualized and defined—and general and nonspecific mechanisms. Among the latter one may include constitution, race, and socioeconomic state. It is with the reports related primarily with this second group of factors that this section is concerned.

Recent studies have contributed little toward a more precise evaluation of the genetic, constitutional, or general familial factor in growth. Blunn observes that rats originating from stocks characterized respectively by a precocious or late growth when bred under identical conditions as to environment and nutrition had offspring which still demonstrated the parental traits. To this not surprising finding should be added Pryor's discussion of the hereditary nature of the sequential pattern of the ossification of the carpus. He believes that such patterns follow the Mendelian laws and illustrates his point with roentgenographs of the hands of triplets and quadruplets. With reference to the growth in multiple births, MacArthur & Dafoe report that the famous Dionne quintuplets are growing

gradually more alike in weight and stature. Somatic size as determined by the number of vertebrae is inherited in swine according to Freeman, whose data show that certain strains have significantly different numbers of vertebrae from others. An interesting reiteration of well-established knowledge is brought out by Barach, who discusses the retardation of stature growth among diabetic children. For a small sample of children that he has observed for several years, he presents data to show in a rather striking fashion that the relative stature of the children is highly correlated with that of the parents. There is also a high intrafamilial correlation with respect to weight at birth as demonstrated by Donald's observations. He has analyzed the birth weight records of some three thousand infants and obtains an intraclass correlation within families of 0.5. Whether this association is due to the common genetic constitution of the sibs or to a similar prenatal environment is not determined. Thoms & Godfried's study on birth weight is pertinent here. They have investigated the correlation between the size of the mother's pelvic inlet measured by Thom's roentgenographic technique and the birth weight of the offspring. Between size of pelvis and birth weight of infant there is a positive although small correlation ($r = .22$). Of the same order is the correlation between birth weight of infant and mother's stature (highly correlated with size of pelvis). The size of the infant is, of course, also related to the size of the placenta. Dow & Torpin have measured this association and find it high. Between volume of sac and weight of baby the correlation coefficient equals 0.69. Probably explainable on the basis of the above mentioned findings is the well-known fact that the weight at birth increases with advancing order of birth. In Meredith & Brown's thorough investigation on the weight of infants from birth to ten days old this generalization is borne out. These authors also note that the immediate postnatal drop in weight is less for light than for heavy infants.

Bearing on the transmission of physical traits from one generation to another are the careful investigations by Schneider (1). This author fed thyroid to *Tribolium confusum* larvae and produced an increased pupal and imago weight. The successive four generations of beetles also demonstrated (but to a diminishing degree) a higher pupal and imago weight although these descendants were never fed, nor came into contact with, thyroid substance or anything except pure flour. In a later paper, Schneider (2) neatly

points out that the transmission of the tendency to increased size takes place through the female. The majority of observations dealing with so-called racial differences reveal known or expected results without elucidating further the elements involved. Dunham *et al.* give data on the weight of Negro and white infants, showing the lighter weight of the former. Steggerda reports on the somatic differences between Negro, Navajo, and white college girls. The most striking differences concern the length of the extremities, superior in the Negroes, and the relative greater width and thickness of the trunk in the Navajos, who constitute the shortest of the three groups. Lall & Townsend have examined the status of the epiphyseal union in Indian girls and find that in terms of skeletal age, Indian girls mature about two years earlier than do Europeans. Probably the most outstanding contribution to the question of racial differences and the effects of the environment is the book of Shapiro. Since Boas's startling observations on the head shape of the descendants of immigrants, the question uppermost in the minds of students on the subject is what significance to attribute to the findings so much at variance with certain accepted anthropological dicta. A first real attack on the problem has been made by Shapiro, who in this book summarizes the results of a well-conceived investigation dealing with Japanese immigrants in Hawaii. He has had measurements taken on samples of (a) Japanese immigrants in Hawaii; (b) their relatives who have remained in Japan; and (c) their offspring born and bred in Hawaii. Analysis of the data shows first of all that the Japanese who have migrated differ in trunk, head, and face measurements from their sedentary relatives in Japan and from their own offspring as well. After taking into account such factors as occupation, sex, age, and place of birth, the author is led to conclude that this sample of immigrants represents a distinct sub-group of the population from which it derives. It could be inferred that in migratory movements there is also physical selection. The differences between the Japanese parents and their offspring born in Hawaii are regarded by the author as effects of the environment, particularly of better economic conditions. The importance of this investigation both from the standpoint of results and of methodology cannot be emphasized too strongly. It initiates an anthropology of migration and should stimulate further studies in a field where knowledge is painfully inadequate.

The differences between socioeconomic classes persist, as newly reported observations indicate. Norman compares the weight, stature, sitting height, and chest girth of English public school boys with those of secondary schools. The age range of the samples is thirteen to nineteen years. As expected, the public school boys are significantly superior in weight and stature. For the other dimensions there is very little difference between the two groups, a fact of some moment. A very interesting series of observations is that by Miller on Newcastle children. The stature and weight of two samples of children five to fourteen years of age from the poorest sections of Newcastle were compared. However, one sample of children consisted of inmates of a children's home where they had been admitted since three years of age. The institutionalized children, although apparently receiving adequate care, were not superior in either stature or weight to the other children. Since the institutionalized children had also less dental caries and a higher blood hemoglobin count, the equality in stature and weight gives much food for thought. In Brockington's paper (1) the relationship between annual growth increment of school children and family income is very apparent. In addition, it is found that the annual growth increment decreases with size of fraternity but only to a certain limit because in large families there are always children who are old enough to work, thereby either increasing the family income or, by moving, reducing the family expenses.

The effects of illness on osseous growth have been studied by Sontag & Harris and by Francis. Sontag & Harris have observed circular striae in the tarsal bones of 63 per cent of infants one month of age. An inquiry into the socioeconomic status, pregnancy, and delivery history of the mothers reveals that these striae may be regarded as signs of disturbed growth reflecting a stormy pregnancy and delivery of the mother. Francis (3) illustrates the characteristics of skeletal growth disturbance associated with disease. There is a lag in the epiphyseal rating (which is his standard) proportional to the duration and intensity of the disturbance. When the convalescence is completed the epiphyseal rating advances. In a later paper Francis (4) presents a comparison of the epiphyseal rating in four groups of infants under one year of age. Breast-fed babies have a somewhat lower epiphyseal rating than infants who are not breast-fed. The lowest is for children who died in infancy; somewhat higher is that for children with a history of gastrointesti-

nal upsets. In diabetic children Bogan finds a retardation in the ossification of the hands and wrists. According to his data, and in agreement with Francis, the retardation is directly proportional to the duration of the disease. Tuberculosis has been generally associated with somatic inferiority, but Potthoff, who examines the stature, weight, and chest diameters of a large sample of tuberculous children two to fifteen years of age, believes that they do not differ from normal children of comparable ages.

The effects of ultraviolet radiation on the weight of mice has been investigated by Ellinger. A reduction of the growth rate is shown, which the author believes is due to stimulation of the thyroid. Luce-Clausen & Brown have observed the growth of three generations of rats under conditions of (a) total darkness, (b) infrared light and (c) visible light. Rats deprived of light grew the slowest, and those receiving visible radiations the best. The important point of this experiment is that these effects were progressively accentuated with each successive generation.

A brief description of the technique for measurement of the optimum temperature for growth in dogs is presented by Baccino. In this note the age curves of the optimum temperature for two kinds of dogs are illustrated. Another aspect of the relation of temperature to growth emerges from Retzlaff's experiments. Male mice caged alone grow less than mice caged in groups of four, eight, or twelve. The author believes that the differences are due to the conservation of heat, the possibility of which becomes greater as the cage becomes more densely populated. The phenomenon was not observed in females, and this negative result is related by the author to estrous disturbances. These conclusions, as the author admits, cannot be considered definitive.

A report which brings out an interesting fact, not well known or given sufficient note, is that of Allen relative to the increments in stature and weight of children during the scholastic year. His data show that at the beginning of each of the English school terms the rate of growth in stature and weight is highest. It is least at the end of the terms. To what extent diet, sunlight, fatigue, or other factors may be responsible the author does not state.

With reference to mechanical factors affecting the growth of the soma or its components, there is a report by Steggerda & Bates on the cephalic index of the Navajos. According to these authors the use of the cradleboard among the Navajos has a very definite in-

fluence on the length of the head and consequently results in an increase of the cephalic index. A study on the difference in size between the right and left upper extremity has been made by Van Dusan. He finds that the superiority of the right upper extremity over the left increases with age and therefore demonstrates the effects of development through use. Relative to this point, Csinady & Szobonya contribute a paper on young athletes. Using photographs they illustrate the asymmetry in somatic development induced by certain types of sports and gymnastics. They recommend swimming as the best corrective exercise.

(c) *Hormones*.—The recent investigations regarding the action of the endocrines on somatic growth have been directed towards a more precise delineation of the functions of the single glands in the complex activity of the endocrine system. On the whole, the observations reported serve to clarify rather than to alter any particular of the established concepts about the somatic growth function of the endocrines. In addition, more data have been accumulated on the action of the hormones on other living creatures besides vertebrates. Lustig & Wachtel (1, 2) in two notes report an acceleration in the germination and growth of cresses treated with anterior pituitary extracts. The details of the experiments in which other substances were also given the plants are not described. In the past such experiments have never been conclusive since it has not been possible to differentiate satisfactorily the nutrient from the hormonal action of the substances administered. However, that hormones can effect the growth of invertebrates has been repeatedly shown and has been recently confirmed by the work of Schneider (3, 4) who fed thyroid substance to flour beetles (*Tribolium confusum*).

To illustrate clearly the relation of the hypophysis to growth, Dandy & Reichert somewhat belatedly describe in detail the experiments made some fifteen to twenty years ago in which Dandy extirpated the hypophysis. The persistence of infantile size together with the appearance of signs of premature senility as the animal becomes older follow the classical description given by Aschner.

Anterior pituitary extract when administered to normal animals leads to a remarkable increase in size according to Freud *et al.* In this experiment acetone-dried powder of bovine anterior pituitary injected for eight months into rats produced animals 10

to 25 per cent heavier than the controls. Kleiber & Cole report that daily injection for six months of an alkaline extract of bovine anterior pituitary substance produced rats ranging from 460 to 500 grams as compared to about 300 grams in the controls. However, when the injections were stopped, the weight of the experimental animals fell. The observations were not conducted long enough to determine if the weight would eventually return to normal once the administration of the hormone was halted. Ross & McLean have investigated whether the increase in weight following administration of anterior pituitary substance is accompanied by an increased skeletal size. More specifically, their question is to find out whether, if under experimental conditions, the long bones resume their growth. From their experiment, conducted on rats aged about six months, it appears that the substance induces histologically recognizable evidences of active growth in quiescent or lapsed cartilage plates and the adjacent spongiosa. In no case, however, was there resumption of endochondral ossification in the articular cartilage. Analogous results, obtained by the Silberbergs (1, 2), are reported in two papers dealing respectively with immature and mature guinea pigs. These authors conclude that anterior pituitary substance has both growth-promoting and degenerative effects on the cartilage. Depending upon the age and development of the animal, the action of this hormone is to stimulate the proliferation of the cartilage and its ossification during parts of the physiologic growth period of the animal and to accelerate the maturation of the skeleton in those animals in which ossification predominates. Similar results are observed by the Silberbergs (3) when this hormone is fed to immature gonadectomized animals. The effects of the castration and of the hormone therapy appear summated and it is interesting to note that in males hyperplastic growth is more accentuated while in females it is the hypertrophic that predominates. Growth in weight and of skeleton are not necessarily concomitant under the same anterior pituitary stimulation. In the guinea pigs observed by the Silberbergs (1) only the skeleton grew. Levie & Uylert extirpated the hypophysis in a sample of rats and in others removed both the hypophysis and the adrenals. Injection of anterior pituitary substance did not halt a decline in the weight of the adrenalectomized animals, but in both groups of animals the same skeletal growth effect was obtained. A note by Lustig & Wachtel (3) relative to the pituitary hormone

is worth recalling here while awaiting further confirmation. The authors announce that by extraction of the anterior lobe of the pituitary with acetone a growth-stimulating factor is obtained which is not Evans's somatotropic hormone. Similarly, they have found a posterior pituitary substance which inhibits growth.

The recent papers regarding the action of the thyroid reaffirm a number of the commonly held views. Grobstein & Bellamy fed thyroid to immature *Platydocilus maculatus* and *P. variatus* and noted, as expected, exophthalmus, a decreased growth rate, and precocious sex maturation. Terroine & Babad report that administration of thyroxin to rats on a full diet rich in protein reduces the weight increment, while increasing nitrogen retention. The Silberbergs (4, 5) have examined the pattern of ossification also in relation to experimental feeding of thyroid substance and potassium iodide to immature guinea pigs. Administration of thyroid hormone causes a slight hyperplasia, a marked hypertrophy, and an accelerated differentiation of the euhyaline cartilage. Ossification is somewhat retarded and there is seen arthropathic rarification of the cartilaginous covering of the joints. These changes are more striking in the females than in the males. Similar findings are reported when potassium iodide was given to the animals. However, this compound is less effective than the thyroid substance in inducing the absorption of bone. Smith & McLean administered sufficient thyroid substance to rats to produce hyperthyroidism and conclude, as the others have, that there results a retardation of growth of the long bones by endochondral bone formation. On the other hand, there was no evidence of failure of calcification in the bones of growing rats, nor indications of decalcification of the bones of older rats. Experiments by Salmon and by Albrecht & Fellingner illustrate very neatly the action of the thyroid on growth in relation to that of the pituitary. Extirpation of the thyroid, followed by administration (Albrecht & Fellingner) or implantation (Salmon) of anterior pituitary substance, did not reestablish growth unless some portion of the thyroid remained. Evans *et al.* have studied further the interaction between thyroid and hypophysis. From their experiments on rats they conclude that gigantism following feeding of anterior pituitary substance is more striking when the thyroid is present, and in the case of thyroidectomized or thyroidectomized-hypophysectomized animals, the growth is maximal when thyroid substance is also added to the an-

terior pituitary hormone. In thyroidectomized-hypophysectomized animals, administration of thyroxin alone does not stimulate growth.

With reference to the action of the gonads on somatic growth, the experiments of Rubinstein *et al.*, published in two journals, reveal that castration of immature male albino rats resulted in a suppression of the growth both in length and weight, and more in the former than in the latter. The animals were castrated at twenty-two days of age and sacrificed at the eightieth. On the other hand, Sandberg *et al.* do not seem to arrive at exactly the same results, at least so far as can be deduced from their statements since they do not report quantitative data. In male rats castrated at seven weeks and six months, respectively, the growth curve of weight up to the age of thirty weeks is similar to that of the intact controls. After that age, the castrates are 10 per cent heavier. In female rats castrated at seven weeks, no difference is noted between the weight curve of the castrates and that of the controls, but the females castrated at six months attain a higher weight level than the controls. Of course, the comparability between the two sets of experiments cited is questionable. More illuminating appear the results published by Bogart *et al.* They examined the weight changes in ovariectomized and intact virgin rats, and rats that were bred. The most precocious weight gain was observed in the castrated animals, the most extensive growth period in the animals allowed to breed. The weight growth of the virgin rats occurred more slowly and for a shorter period than that of the others. The authors believe that additional confirmation is thus given to the view that estrogens inhibit growth while the corpus luteum stimulates it. The Silberbergs (6) have given estrogens to immature guinea pigs and observe a "premature aging" of the cartilage and replacement of bone but no remarkable change in weight. Moracci reports that the administration of corpus luteum to tadpoles causes these animals to become heavier and to metamorphose earlier than others. It will be recalled that Kopeć (*vide supra*) showed that the daily weight curve of female rats proceeds in waves. This was not found true for the males. The finding has been investigated further by Adamska who notes that, in ovariectomized animals, the growth proceeds as in the males. This result is also interpreted as an indication of the inhibitory action of estrogens on weight.

According to Sainton *et al.*, the prolonged subcutaneous injection of testosterone propionate to immature rats decreases the growth rate of the body weight and length, and weight of the thymus and testicles (but not that of the seminal vesicles). Similar results are reported by Rubinstein *et al.* who note, moreover, that while castration (in their experience) produces a greater relative inhibition of length than weight, the reduction brought about by testosterone propionate is proportionally the same for both somatic variables. The observations on man made by Kenyon *et al.* differ from the above experiments. Intramuscular injections of testosterone propionate given to normal men and women as well as to eunuchoids were followed by an increase in weight. McCullagh & McGurl also report the same and emphasize, in addition, that this substance when given in sufficiently large doses may bring about precocious epiphyseal maturity.

What effect the thymus exercises on somatic growth remains still an open question. Although Comça gives some data to show that thymus extract increases body weight of guinea pigs and produces some decrease in size and development of the testes, Chiodi (1) who has repeated Rowntree's experiments obtains completely negative results. Neither did thymectomy in five successive generations of rats produce any appreciable change in growth and development according to Chiodi (2). No growth-promoting effect was observed when thymus substance was fed to rats by Segaloff & Nelson, to mice by Smith & Jones, or to the larvae of *Rana pipiens* by Janes & Segaloff.

At various times a growth function has been attributed to the pineal gland. Einhorn & Rowntree have conducted experiments to elucidate this problem and their findings indicate that pinealectomy in successive generations of rats does not influence the growth of the offspring, nor was the growth in pinealectomized rats affected by intraperitoneal administration of pineal extract. However, the daily administration of this extract to four successive generations seems to have brought about some retardation of growth, progressive in each succeeding generation.

(d) *Nutrition and vitamins.*—The studies on nutrition and vitamins in relation to growth reflect a variety of approaches and objectives; until a systematization of the daily discoveries and pseudo discoveries is effected, it will be difficult for the time being to obtain a clear view of what is really being accomplished.

The affects of temporary and partial starvation have been examined further by Jackson. Underfed rats, when compared to young animals of the same weight, are superior in size of skeleton, eyeballs, stomach, kidneys, and testes. They are inferior in size of thymus, liver, and spleen. When the underfed rats are returned to an adequate diet *ad lib.*, a rapid increase in growth follows. This growth increment affects each of the organs in such a fashion that the usual proportionate size is attained. The somatic characteristics of starvation appeared in the rats to which Wilson *et al.* fed a diet containing nicotine. Histological examinations of the organs revealed no sign of toxic damage by nicotine; accordingly, the authors concluded that the starvation effect was produced by inanition. These observations bring out an essential and elementary point, sometimes overlooked in feeding experiments, that a growth decrease may be wrongly attributed to the lack of some substance instead of to the reduction in food consumption.

The search for specific growth-promoting factors in foodstuffs continues to be the most exciting problem to engage the attention of investigators. Jukes & Babcock's report has called attention to a water-soluble antiparalytic factor in alfalfa that improves the growth of chicks. Stokstad & Manning describe in detail the chemical properties of what they have called factor U, which promotes growth in chicks. This factor is present in alfalfa, middlings, wheat bran, and yeast. Its physical and chemical characteristics are unlike those of any other factors so far described.

More information on the growth action of the proteins and amino acids appears in recent reports. Observations by Robinson *et al.* emphasize the growth-stimulating effects of chondroitin-sulfuric acid added to the basal diet of chicks and rats. However, in a later publication by the same group of authors, Crandall *et al.*, it was brought out that this effect is dependent on the fact that their basal diet was not adequate. For example, with chicks on a diet producing gizzard erosion, the addition of the chondroitin-sulfuric acid neither improved growth nor reduced the frequency of gizzard erosion. When rats are fed a diet sufficient in sulfur protein, the addition of cystine does not improve the growth, according to Haag & Wright, but the addition of methionine does. On this question the observations of Rose & Rice appear rather definitive. They placed rats on diets lacking cystine and methionine. After the expected decrease in weight was observed, methionine was added

and the growth curve took its normal upward way. However, with the addition of various forms of cystine, the weight either continued to decrease or increased at a subnormal rate. The authors conclude, therefore, that cystine is not a constituent of food essential to growth, and that methionine is the only indispensable sulfur-containing amino acid. Other aspects of the action of cystine and methionine are discussed by the Whites and by Stekol. The Whites report that when cystine or methionine was added to the diet of rats fed methylocholanthrene, the inhibitory effects of this substance on growth were promptly overcome. Their findings also suggest that methylocholanthrene produces a deficiency by involving the sulfur-containing amino acids in the detoxication of the hydrocarbon. Stekol fed phenanthrene to growing rats and their growth ceased. When cystine and methionine were added to the diet, the growth was resumed. Stekol arrives at the same conclusion as the Whites. More specifically he believes that phenanthrene is detoxicated in the rat to yield a mercapturic acid which is responsible for the growth inhibitory effect of phenanthrene. An extensive and thorough study of the growth effects of a deficiency in cystine and lysine has been published by Lafon, who has summarized a series of well-conceived experiments, adequately analyzed. The arrest in somatic growth (in length and weight) of rats and mice on a prolonged diet which is deficient either in cystine or lysine is not accompanied by an arrest in the growth or development of all the body organs. The brain, kidneys, and eyes progress in size, while the physiological processes such as the involution of the thymus, ossification, and sexual maturity follow their time schedule. Moreover, neither the anterior pituitary hormone nor thyroxin reestablish the growth of the animals on such a deficient diet, which shows that the dietary deficiency does not produce pituitary or thyroid insufficiency.

On the protein requirements of diet, Hamilton's paper is of interest. His experiments show that an indefinite increase in the protein component of the diet of rats does not lead to a continuous increase in the growth of the animals. The growth-promoting effects of the diet augmented as the percentage of proteins in the diet rose from 4 to 16, remained constant when the percentage was 16 to 22, and decreased thereafter. In man, the similar general observations by Brockington (2) are of interest. From data about the food expenditures of the families of children whose annual weight

growth was measured, he determined the ratio of the annual expenditure for protein foods to that of the expenditure for energy foods. He finds a low, but significant, positive correlation between the gain in weight and the proportion of animal protein foods bought. This positive correlation could be demonstrated for all levels of total food expenditure.

Marquis has investigated the effects of a diet almost totally lacking in chlorides and reports in a brief note without sufficient details that the growth in weight of rats was arrested. When the animals were returned to a usual regimen after a period of such diet, a rapid growth was observed. Lack of magnesium also produces an arrested growth. According to Duckworth *et al.* there is a fragility of the bones due to the depletion of skeletal magnesium, and a decrease of weight. Restoration of this mineral to the diet brings with it the normal increase in weight. The effects of phosphorus deficiency are dramatically shown by the observations of Day & McCollum that rats subject to such a diet died rapidly and, of course, without achieving much growth. Bearing on this element is the study of Gaunt *et al.* on three generations of rats kept on a diet poor in calcium and phosphorus. In growth rate and reproductivity these rats showed a distinct inferiority which became more marked in each successive generation. When calcium and phosphorus were added to the diet, improvement was noted which, it is important to note, assumed about the same characteristic whether these minerals were provided in the form of salts or in vegetables and milk.

Fairbanks fed rats bread samples containing 6 and 12 per cent of milk solids, respectively, and reports a definite increase in the length and weight of the animals as compared with animals fed plain bread. He attributes this increase to the greater concentration of protein, calcium, and phosphorus in the experimental bread. The results of feeding calcium lactate or skimmed milk daily for eleven weeks to Indian children on a poor diet are described by Aykroyd & Krishman. The net effect of giving either 1 gm. of calcium lactate or 8 oz. of skimmed milk was that the experimental children gained in weight about 1 lb. more than did the control, and in stature about 1/5 inch. When newborn infants are given a complementary diet, the loss of weight is reduced, as Riesenfeld points out. His observations on over one thousand infants indicate that the greatest postnatal weight loss occurs in

breast-fed babies. Sanford's data would indicate, however, that breast-fed babies regain their birth weight at a more rapid rate than others. This whole question deserves more critical study, since it is not only the health of the baby but that of the mother as well that is involved.

With reference to vitamin D specifically, an elaborate experiment by Nicolaysen & Jansen on rats should be mentioned. These authors sought to determine whether calcium and phosphorus could be substituted for vitamin D as antirachitic agents. Their observations seem to indicate that it can. The effect on the skeletal growth of supplementing the diet of children with milk and with milk plus vitamin D are reported by MacNair & Roberts. As expected, the addition of one pint of milk per day to the diet of institutionalized children resulted in an acceleration of skeletal growth as determined by roentgenographs of the wrists. However, the growth response of the children who received the milk plus the vitamin D was not significantly different from that of the children who received milk alone. MacNair examines also the skeletal growth records of a sample of institutionalized children who were given daily a small dose of cod-liver oil. At the end of a year some acceleration in the growth of the experimental children as compared to the control group was observed. An important investigation on the subject is that by Jeans & Stearns which, unfortunately, is not based on a large sample. These authors have compared the rate of linear growth in the first year of life of infants given from 1,800 to 4,600 units of vitamin D daily with the standard published rates and with the growth of infants who received only 135 to 340 units of vitamin D. The growth of the first group of children was similar to or slightly less than that of the last group. The authors believe that to obtain the optimum effects no more than 340 to 600 units daily need be given.

New sources for substances whose actions correspond to those of the vitamin-B complex have been investigated. Randoin *et al.* report that in eel skin and in the pigmented portion of carp scales a flavin substance is present. Their conclusions are based on experiments on the growth of rats fed a vitamin-B₂ deficient diet. The rats which received a supplement of powdered eel skin grew even more rapidly than those receiving a supplement of lactoflavin. Stirn *et al.* give evidence to show that, so far as growth is concerned, natural fats or synthetic fatty acid esters can replace thia-

min. To rats on a diet lacking thiamin but complete in all other respects, they gave fats for six weeks after the symptoms of polyneuritis appeared. The rats recovered and grew at the usual rate. It is noticed, however, that on such a diet the animals were unable to build reserves in the liver although they obtained enough energy for adequate growth. Hitchings & Subbarow have experimented further to determine the growth factors present in the "95 per cent alcoholic liver filtrate." Observations on growth after a series of extractions, lead them to the conclusion that pantothenic acid is the growth-promoting substance. The observations of Hoffer & Reichstein, briefly reported, would indicate in addition that β -alanine is the essential part of the whole molecule of pantothenic acid since, relative to growth, rats react in a similar way to both substances. In some disagreement with the above are the results of the experiments by Oleson *et al.*, only summarily described. These authors note that the growth stimulation due to β -alanine alone was not significant and that the intact pantothenic acid molecule is required. However, this factor will not completely replace the original liver extract or crude filtrate. Therefore, the factor W is also needed. From the fact that vitamin-B deficiency produces hydremia, Litchfield *et al.* were induced to study whether the action of this vitamin in fluid retention would be useful in the care of premature infants. They have treated fifty-eight premature infants with yeast extract and report that after the first week 55 per cent of these infants had begun to gain weight as compared to only 10 per cent of a sample of untreated infants. Treated infants, with birth weight less than 1,500 gm., attained four to five times their birth weight in three months. During the same period untreated infants reached only two to three times their birth weight. Rather striking effects associated with the feeding of vitamin B₁ and iron to children are reported by Summerfeldt & Ross. Institutionalized children fed a 3 oz. daily ration of a cereal rich in vitamin B₁ and iron showed remarkable gain in weight when compared to other children. The study is, however, based on a small sample.

With the progress of investigations in the field of vitamins and endocrines, some evidence has accumulated that probably the activity of one group of factors may effect the action of the other. Thus, Drill has recently shown that the decrease in weight produced by the administration of thyroxin may be overcome, partially at least, by administration of both vitamin B₁ and B₂.

Perters & Rossiter find the same to be true and on the basis of more intensive chemical analysis interpret the findings as indicating that the giving of thyroid substance changes the animal's normal requirement for vitamin B. It may be then that the variations in response to vitamin-B feeding rest on an endocrine foundation.

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TEMPERATURE REGULATION

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This review deals with some of the literature from August 1938 to July 1940 and a few later papers.¹ Earlier literature was considered by Burton in Volume I of this series. The period here under discussion is not marked by any major advance in either technic or theory. There has, however, been a steady progress in certain phases of the subject. This has been particularly true in the analysis of the parts of the brain involved in thermal control.

THE CENTRAL NERVOUS SYSTEM AND THERMAL CONTROL

The importance of the hypothalamus in the regulation of temperature is now unquestioned. Evidence is accumulating that the reactions to heat and cold are to some extent dependent on two distinct centers separated from one another in the brain. Such a concept does not deny that in the intact animal these centers are coordinated reciprocally to form a single mechanism. It merely states that separate parts of these reactions are carried out in different situations in the brain so that experimental or pathological lesions may remove or impair parts of the total reaction. Large lesions of the hypothalamic region may markedly impair or destroy all temperature regulation; smaller lesions allow differentiation to be detected.

The ability of cats to regulate their temperature when tested in a cold or hot box was examined by Ranson and his associates both in normal cats and in others with hypothalamic lesions (10). Large central lesions dorsal to the optic chiasma and ventral to the anterior commissure may cause serious impairment of the heat-dissipating reactions that prevent overheating, with little damage to the reactions causing heat conservation and increased metabolism that prevent chilling. Bilateral lesions in the lateral parts of

¹ Due to limitation of space, it has been impossible to discuss all relevant papers, but a few that seem especially pertinent have been included at the end of the bibliography although they are not discussed in the text (references 110 to 123).

the anterior hypothalamus have a severe effect on the reactions to heat but also produce moderate impairment of reactions to cold. Bilateral lesions in the caudal part of the lateral hypothalamus cause marked impairment of both reactions.

Impairment of the reactions to heat include decreases in both polypnea and sweating from the pads of the feet. Impairment of the reactions to cold is indicated by reductions in rectal temperature which exceed those of the normal animal. Circulatory responses were not tested specifically. One may assume that if the fall of rectal temperature on exposure to cold was not abnormally great, vasoconstriction was probably normal, since no evidence of exaggerated shivering was reported. Thalamic lesions were also made in some animals (11), which were put to similar tests. These animals were also examined at the end of the period of survival for their capacity to show a panting reaction after acute decortication.

It is concluded that a center for reactions to heat is situated in the anterior hypothalamus, and one for reactions to cold in the caudal part of the lateral hypothalamus. Lesions limited to the medial parts of the hypothalamus in the regions of the infundibulum and of the mamillary bodies do not interfere with either center nor with their tracts. Damage in the caudal part of the lateral hypothalamus is considered to injure not only the centers reacting to cold but also motor paths from the centers reacting to heat. These fibers are considered to descend in the medial forebrain bundles of the lateral hypothalamus. Some of the abnormalities observed might depend on interference with sensory impulses normally acting on either center. No method has been devised to put such an hypothesis to the test. While the polypneic response to heat appears to arise from the center in the anterior hypothalamus, this center is not essential. According to previous workers polypnea can still be obtained after removal of this center if the caudodorsal portion of the thalamus is intact. Any such center in the thalamus is also not essential. No lesions in the thalamus were able to prevent the response if the hypothalamus was intact (11).

The possibility of the existence of more peripheral thermoregulatory mechanisms which can be active after high spinal sections is discussed by Ranson (4). Work from his laboratory shows that slow acclimatizations to temperature can develop in spinal cats. These are probably metabolic adjustments of hormonal origin (9). Variations in vasomotor tone from the direct effects of tem-

perature on the vessels are not excluded. No indication was found of regulatory control by the spinal cord. Contradictory results reported by Thauer (7) are considered due to incomplete sections (4).

The mechanisms involved in the stimulation of thermoregulatory centers are still undetermined. It has been assumed that thermal changes within these centers are dependent on the temperature of the inflowing blood while the thermal effect of the metabolism of the centers themselves is negligible. Evidence is now advanced that such assumptions are unwarranted. Serota (21), by the use of thermocouples inserted with aseptic precautions in different areas of the brain, finds that the temperature in the hypothalamus is consistently 0.5° to 1.0°C. higher than that in the cortex. During sleep the temperature falls in both, but more in the hypothalamus than in other regions. This fall in the temperature of the hypothalamus is not dependent on variations in the rate of blood flow as determined by Gibbs' thermojunction method. The metabolic level is presumably high. The effects of heating the anterior and posterior regions of the hypothalamus have been investigated (15). Small diathermic electrodes were inserted aseptically so as to allow heat to be concentrated on a small area. Warming of the anterior region caused vasodilatation of the ear and the abolition of any shivering which might happen to be present originally. Panting was not seen. Warming of the posterior region of the hypothalamus produced no vasodilatation but did induce sleepiness. Conditions were somewhat different from those of Ranson's experiments. Changes in the temperature of the centers would be associated with a different pattern of sensory stimulation.

The effects of dinitrophenol and of typhoid vaccine have been tested in cats with hypothalamic lesions (20). The pyrexial response to the former of these appears normal; that to the latter is usually atypical or absent. Often the vaccine produced a fall of temperature lasting twenty or more hours. One must await data obtained by methods such as those of Serota (21) to develop the theoretical significance of such observations. The hypothalamus also seems concerned with the regulation of temperature by hormones, and by fluid and salt adjustments in the blood. In the rat an increased activity of the thyroid in response to cold is dependent on connections from the hypothalamus to the anterior pituitary (22). Barbour (8) finds that exposure to cold causes a blood concentration with increase in the specific gravity of the plasma and

also an increase in its total osmotic pressure. The change is one associated with a reduction in blood volume and therefore with a similar reduction in the vascular bed. The changes are dependent on the integrity of the anterior hypothalamus, an area in other respects associated with responses to warmth rather than cold. Barbour regards these changes as due to a shift of water into the cells and considers vasomotor adjustments secondary to them. The reverse may be true. The increased salt concentration of the blood and lowering of vapor pressure would assist heat conservation, but the effect is insignificant.

Neurogenic hyperthermia in man as a symptom of neuropathology is usually but not invariably associated with lesions of the hypothalamus demonstrable at autopsy (14). Similarly hypothalamic degeneration is found in patients who have died of heat stroke (17). Disturbances of thermal control accompanying lesions of the central nervous system in man are described and discussed (12, 23). Recent reviews have appeared dealing with the complicated physiology of the hypothalamus (5).

The effect of pronounced cold in depressing nervous reactions has long been known. It is found that cold also depresses the electrical activity of the cortex, posterior hypothalamus, and medulla; both the frequency and amplitude of the electrical changes are reduced (16). Q_{10} when measurable has values between 2 and 2.5.

Little is known of the development of the thermoregulatory mechanism. The human child at birth has a comparatively inefficient mechanism; the variations in temperature under normal conditions, are however, slight (1°F.) and irregular, with little evidence of any definite diurnal rhythm. From the sixth month the temperature curves become progressively more regular and the total changes in temperature consequently greater (18). By the eleventh month the variations normally have a range of 3°F.

The sensitivity of the centers to changes in body temperature varies with the conditions. Under normal conditions an increase of 0.1°C. in rectal temperature causes polypnea in rabbits; with dehydration the threshold is increased to 2° or 3°C. Giving the animal water lowers the threshold. Intravenous injection of hypertonic saline has an effect similar to that of dehydration, while injection of hypotonic saline produced an effect similar to hydration (13). The rise of rectal temperature during exercise is greater the heavier the work. It depends on a readjustment of regulation, rather than

on impaired heat loss, for the rise of rectal temperature is the same whether the subject works in a room at 5°C. or at 23°C. (19).

RECEPTORS AND SENSATION

Experimental investigation of temperature sensation continues, but the inherent difficulties of analysis of sensation are still present. Jenkins has improved Dallenbach's original method for mapping cold or warm spots and emphasizes the use of a seriatim method where the ultimate data are analyzed statistically (31 to 35). It may be mentioned that in principle this procedure is not new, since repeated testing of sensitive spots has long been employed by the more careful workers. His careful study has produced new information. Repeated tests of the same area may give minor variations but are remarkable for consistency, with correlation coefficients of 0.85 or more. Applicators of different sizes have been used and the intensity of the resulting sensation has been estimated. The point is made that the sensation resulting from the stimulation of a given area is less than the sum of those from separate stimulations of parts of that area. The relationship is such that the sensitivity of the total area stimulated at one time is equal to the square root of the sum of the squares of the scores representing the sensitivities of the various parts. The method of scoring is entirely empirical, yet the agreement of the data with this general statement is surprisingly good. Jenkins concludes that these data are not explicable in terms of a few isolated receptors and diffuse thermal conductance in the sensitive areas. He believes that no discrete sensitive spots exist, but merely indefinite areas of greater or less sensitivity. Emphasis is placed on sensations of temperature from stimulation of a part of an area, which may exceed in intensity sensations generated by stimulation of the whole area. He states for instance (34) "a single receptor which is responsible for two part scores" (i.e., stimulation of parts of an area) "cannot conceivably be stimulated less strongly by a stimulator which covers both parts." Such generalizations are unwarranted. Unless the inflow of water into the applicator is very high the actual strength of the stimulation is apt to be lower the larger the area to which it is applied. This depends on the greater heat capacity of the larger area. The uniformity of the stimulus should be demonstrated by thermocouples, before sweeping deductions are made. Even with a constant stimulus the results obtained might be explained on the basis of a few dis-

crete spots, if stimulation depends on the steepness of thermal gradients. He invokes hypothetical receptors scattered in large numbers over the area, which are considered to give sensation in proportion to the square root of the number of receptors stimulated. His interesting data do not justify such a sweeping assumption of receptors not demonstrated among those that have been recognized histologically.

Jenkins (30) also denies the necessity for a paradoxical cold sensation as an element in the production of a "hot" sensation. This is in contradiction of a theory originally proposed by Alrutz (24). In the opinion of these reviewers the data do not exclude an element of cold in the normal hot sensation. It was recognized long ago by such classical masters as Head and von Frey that the subjective interpretation of mixed sensations is difficult. A hot stimulus can without question excite receptors for warmth, cold, and pain. It is probably that the subjective sensation "hot" will be reported by many individuals from varying combinations of these or even from a sensation of pure pain alone. Thus, speaking of pure pain sensations, Head (29) wrote: "Any ordinary patient would have called such stimuli hot because the pain produced is of a kind associated in daily life with the action of hot bodies only."

The response of cold receptors has also been studied by directing air of a known temperature against the face for varying periods of time (25). Liminal intensity values plotted against time gave a descending hyperbolic curve. The experimental work of Hardy & Oppel on induction of sensations of cold by radiation was reported in the earlier review by Burton; since that date this work has been reported in greater detail (27).

Spatial summation is seen for cold (27) as well as for warmth (26), so that the stimulus required is less when a large area is stimulated. With stimulation of areas 5 sq.cm. the threshold for warmth and cold are the same; with larger areas, that for cold is greater than that for warmth, with smaller areas less. The data are interpreted as consistent with the view that the cold spots are more numerous than warm spots, if spatial summation is less developed for cold than warmth. Whereas stimulation for three seconds was necessary for warmth, only one second was required for cold. The data agree well with those of Bazett & McGlone which indicated cold receptors to be more numerous and more superficial than warm receptors. The threshold for cold sensation when a large area

is stimulated is a decrease in temperature of 0.004°C . per second on the surface, a value about four times as great as that for warmth. The curves relating the threshold to the area stimulated when extrapolated agree with those found for spot stimulation by Bazett & McGlone. While the change in surface temperature was found by the latter workers with spot stimulation to be less for cold than for warmth, they believed that the actual threshold change in temperature at the depth of the receptor was greater for cold than for warmth. Spot stimulation is relatively inefficient for the less superficial receptors for warmth. The agreement with the data of Hardy & Oppel is therefore close.

The stimulation of pain by radiant heat has been examined quantitatively (28). The threshold for producing pain is a change in temperature about fifteen hundred times greater than is necessary to induce sensations of warmth. There is no evidence of spatial summation in estimations of pain intensity. The threshold for rapid production of injury with blisters is only twice the threshold for pain. Since acetylsalicylic acid lowers the threshold for warmth while increasing that for pain, and since the two sensations are also differently affected by ischemia, the peripheral structures involved must be different.

PHYSICAL REGULATION

Partitional calorimetry.—This procedure in which the heat loss is analyzed into its various components, received attention in the previous review. The same investigators have continued their studies in this field, applying the method to the clothed subject (50, 76, 77, 78), and developing a temperature scale for use under all conditions (49). Loss of heat by evaporation is determined, and the remaining loss is partitioned into that by radiation and convection. The conditions for the total loss by the latter means are expressed in a composite "operative temperature" which depends on air temperature, wall temperature, and air movement. The "standard operative temperature" of a room in which the wall and air temperatures are identical and the air movement is at a standard rate is the dry bulb temperature of the room. When the wall and air temperatures are different, or the rate of air movement is changed, the standard operative temperature (T_0) represents an imaginary environment with the above standard relationship in

which the subject would lose the same amount of heat by radiation and convection without a change in surface temperature.

Operative temperature partitions heat loss by radiation and convection. These losses depend on the size and shape of the surface of the individual; the radiation and convection constants vary for different individuals and vary even with the posture in the same individual (78). Where wall and air temperatures differ, the operative temperature of the room for one subject is not identical with that for another subject. The addition of clothes alters these constants from those of the nude subjects. The radiation constant is little changed; radiation from the skin to the clothes is continued by radiation from the clothes to the environment. The convection constant is much lowered since air movement is considerably reduced. Evaporation of water continues, and acts as though the evaporation under ordinary conditions still occurred at the skin surface. In a room with standard air movement and with the air and wall temperatures identical, the ratio of heat loss by convection to that by radiation with a resting nude subject is of the order of 1.09; with the subject clothed it is of the order of 0.80. The change is due mainly to the reduction in convection losses. At operative temperatures between 25° and 29°C. the resting clothed subject can regulate heat loss by vasomotor adjustments; below 25° body cooling results (as compared with a limit of 29° for the nude subject); above 29° evaporative regulation is required. Analysis of the regional variations in changes in skin temperature on exposure to various conditions for both clothed and nude subjects is given. The conductance of the clothes is estimated; the latest estimate (78) is 7.3 kilocal. per sq. m. per hr. per degree C.

With cold air and warm wall conditions, skin temperatures and skin conductance are lower than for similar operative temperatures when the air and wall temperatures are identical. The wetted area of the skin is also less. Vasoconstriction in the skin is definitely greater. The possibility of these differences being dependent on some reflex effects of temperature sensations in different parts of the body is discussed. The effect of air movement on convection losses is shown to vary with the square root of the air velocity (78). While the gross responses of the nude and clothed body are similar, the upper limits of regulation of the clothed subject are greater provided that atmospheric humidity is low. The upper limit is 52°C. for the clothed as compared with 43°C. for the nude subject.

This depends on the protection of the body by the clothes from heat inflow from the environment. At high humidities the clothed subject adjusts less well than the nude.

The use of the standard operative temperature scale is intended for application to more general conditions where the calorific loss is known without analysis of its partition (49). Thus the symbol K_0 is used to denote the standard rate of heat loss by convection and radiation for a 1°C . difference in temperature between the skin and the environment as measured in terms of T_0 as already defined. Then,

$$\text{heat loss} = K_0 (T_s - T_0)$$

where T_s is the average (weighted) skin temperature. Consequently

$$T_0 = T_s - \text{heat loss}/K_0$$

If the surface temperature and heat loss are known, any conditions, such as baths, may be expressed in terms of the equivalent operative temperature.²

Total loss by radiation, convection, and conduction.—Hardy & Soderstrom (54) conclude from calorimetric observations that vasomotor effects are insignificant in the regulation of heat loss at environmental temperatures below 28°C . for the nude subject, while from 28° to 30° they play an important role. However, others have considered vasomotor tone as not constant even at lower temperatures (76). Above 30° evaporation is important. The skin acts as a perfect (98.9 ± 1 per cent) black body for infrared rays (52). Heat loss by radiation, convection, and vaporization during shivering, in chills produced by exposure to cold or by reaction to malaria, is essentially the same as with exercise (53). The importance of radiation in the home and the problems of regulation of room conditions in the tropics have been investigated (45, 66).

The principles of operative temperature and measurement of skin circulation by estimating the thermal conductance of the tissues have been applied to animals by Herrington (56). The smaller animals with their relatively large ratios of surface to body weight have only incomplete regulation of rectal temperature. The insulation of the guinea pig is much superior to that of the rat. Estimates of the surface temperatures, mean body and rectal

² The further development of the preliminary account has now appeared: *American Journal of Physiology*, **131**, 93 (1940).

temperatures, and predictions of metabolism for varying external temperatures are given for guinea pigs, rats, and mice. Thermal neutrality zones are 30° to 31° for the guinea pig, 28° to 29° for the rat, and 30° to 33° for the mouse. Standardization of thermal reactions in dogs has been reported by utilizing slow cooling of the animals in a constant room environment and determining the amount of heat which must be given by diathermy to remove the reactions to cold (55). Control of radiation and convection losses is attained by alterations in surface temperature induced by vasomotor adjustments, though evaporation also has a major effect on skin temperature. Burton (42) has described an apparatus for the direct measurement of the conductance of superficial tissues. The circulatory adjustments will be referred to later.

Heat loss by evaporation.—This loss depends on evaporation from the skin and respiratory tract. Sensible sweat is entirely of glandular origin. The term insensible perspiration is used here to include evaporated capillary filtrate and insensible sweat. Insensible loss of weight by water evaporation in the rabbit is unaffected by a reduction in atmospheric pressure of the order of 200 mm. Hg (68). The insensible weight loss of children has been investigated during sleep (46). There is an initial transitory rise followed by a considerable reduction which parallels the fall in rectal temperature. The data are consistent with the hypothesis that rectal temperature falls because of a reduction in metabolism, which is accompanied by a parallel reduction in water evaporation. It is concluded that a period of at least four hours should be utilized for estimations of metabolism in sleeping children by determinations of weight loss. Even so the data may be misleading. In the discussion of slow adaptation to temperature later in this paper it will be seen that conditions can arise in which the normal relationship of water loss to metabolism is considerably disturbed.

Abundant evidence continues to be obtained that cooling is largely dependent on water evaporation during the high metabolism of exercise or on exposure to high temperatures (19, 54). As a result, during exercise there may be little or no increase in surface temperature in spite of a marked rise in rectal temperature (19). At ordinary room temperatures evaporation at the skin surface is little affected by variations in relative humidity (from 35 to 75 per cent). Consequently the skin temperature depends mainly on the environmental temperature (70). If higher humidities are utilized

(90 per cent) effects on skin temperature are reported (48), particularly in the extremities. Modifications of the sweating reaction develop with slow adaptations; these will be discussed later. It may be remarked that it remains uncertain whether insensible perspiration of water continues during pronounced sweating; it seems improbable. According to old experiments by J. S. Haldane (74), water transference across the cuticle in insensible perspiration depends on the difference in water vapor tension on the two sides of the cuticle. As he pointed out, if the external surface of the cuticle is wet with sweat of a low saline concentration, the vapor tension on this surface should be slightly greater than that of the plasma. Water movement should be slight and in the reverse direction. With heavy sweating probably all the fluid evaporated is derived directly from sweat glands.

Local faradic stimulation of the volar surface of the forearm elicits secretion of sweat over an oval area of some 3×5 cm. (75). The rate of secretion, but not the number of glands affected, is greater when the skin is warm. Secretion depends on the presence of sympathetic fibers for it is absent after removal of the sympathetic ganglia. Preganglionic fibers must be present to obtain reflex sweat secretion but are not essential for this effect of direct stimulation. The presence of numerous interdigitating axon systems, with a single gland receiving secretory innervation by branches of several axons would explain the data obtained. The local effect may be blocked by novocaine and by atropine and enhanced by prostigmine. Similar data were reported by Bickford (39). Injection of acetylcholine intradermally can stimulate sweat glands to secretion (44). In larger concentrations this results from a direct muscarine-like action in which the effect spreads slowly from the point of injection and is not antagonized by local anesthetics. With small doses the action of acetylcholine resembles that of nicotine. Injections of 0.1 cc. of 1/100,000 nicotine, 1/1,000,000 α -lobeline or 1/40,000 acetylcholine induce secretion of sweat simultaneously from glands extending over an area of 4 cm. diameter. An axon reflex is apparently evoked; the receptor end behaves like an autonomic ganglion; the effector is cholinergic. This effect is antagonized by 1/200,000 novocaine. A study of the loss of sodium, chlorine, potassium, and water in sweating of subjects maintained in mild pyrexia by heating for periods up to forty-eight hours has been made (59). There may be a loss of 7 to 19 per cent of the total

sodium chloride of the body. The sodium content takes several days to recover the normal value and then rises above the initial level. Some loss of potassium may also occur in pyrexia.

Respiratory loss of water occurs mainly in the nose and throat but probably partly beyond this area. Measurements of temperature and water vapor tension in the nose and nasopharynx during both inspiration and expiration are reported under varying room conditions (72). Under ordinary conditions (with a humidity of 35 per cent), air at the posterior margin of the inferior turbinate is said to have a temperature of about 31°C. with humidity of 86 per cent in inspiration and a temperature of 33.5° with humidity of 79 per cent in expiration. Breathing air at 8°C. can apparently cause temperatures at this point as low as 27° during inspiration and 32.5° during expiration. Loss of heat by vaporization in the panting reaction of dogs has been quantitatively examined (73). Water loss is increased to between eight and ten times the basal value. In spite of the shallow respiration some washing out of carbon dioxide occurs. During the first half hour the respiratory quotient may be as high as 1.3; it gradually falls during prolonged polypnea to 0.81 after 3½ hours, as alkalosis reduces the carbon dioxide tension in the blood. Polypnea is accompanied by a considerable increase in oxygen usage, amounting to 50 per cent to 100 per cent. This may perhaps be related to the high oxygen consumption associated with voluntary hyperpnea in man as compared with an equivalent hyperpnea produced by carbon dioxide as was reported many years ago by Liljestrand (61). The common factor may be alkalosis.

The pH of the blood, as measured by a glass electrode, has been determined regularly at six-day intervals in dogs as well as in human subjects to determine seasonal changes (37). It is concluded that the two series show a close correlation with each other and that there is a statistical correlation of pH changes with variations in the barometer and to a less extent with the environmental temperature. The weekly averages were found to vary from 7.28 to 7.68 in man, and from 7.32 to 7.68 in dogs. It seems improbable that small changes in barometric pressure were causally related to such large changes in pH. A study of the pH of blood in the tropics has also been reported; some evidence was obtained that the pH was slightly higher there than in a more temperature climate. The pH of arterial blood was 7.44 ± 0.0048 in residents of European origin (69).

Circulatory changes.—The importance of vasomotor control and of the rate of blood flow in the control of heat loss is well recognized. In acute exposure to heat it has been shown that increased cardiac output, peripheral dilatation, and increased blood volume (up to about 10 per cent) are all correlated in such adjustments (58). The dilatation impairs the capacity of the subject to adapt to changes in posture (60, 65). The changes in the circulation may be associated in severe exposure to heat with inadequate venous filling and a reduction in blood volume due to excessive sweating (58). In anesthetized rabbits exposure to warmth causes an initial fall and a later rise in blood pressure, with an initial reduction and a later rise in the effective peripheral resistance. The stroke volume is reduced throughout (40). Such data on rabbits duplicate closely those previously reported on man from our laboratories. Perfusion of an isolated carotid body with oxygenated blood at 15° and 45°C. gives both increased respiration and a rise of blood pressure at the higher temperature (38). The rise in pressure is attributed to vasoconstriction. On the other hand perfusion of the separated carotid sinus area with heated saline is said to give vasodilatation (40).

The extremities play a major role in the control of heat dissipation. Quantitative estimates of blood flow in the fingers and toes have recently been reported by Burton (41), who finds flow in a finger to range from 1 to 90 ml. per 100 ml. of tissue per min. Rhythmic variations in flow occur, usually developing simultaneously in different areas. In a later paper (43) he concludes that the rhythmic change in vascular tone is dependent on the state of thermal equilibrium. Control is exerted not only by the degree of vasoconstriction, but also by the rhythm of waves of vasoconstriction and the resulting relationship of periods of constriction to those of dilatation. Sudden changes in this rhythm appear correlated with alterations in the rate of change of deep body temperature. According to some workers (65, 71) the temperature of the toes is increased when the extremity is in the pendant position. Others (62) report a marked fall and assume that the disagreement is due to the degree of tilting of the subject. All agree that there is a general fall in skin temperature in the upright posture. This may be regarded as a compensatory vasoconstriction to a decrease in venous return and cardiac output. Any contradictory increased flow through the toes may be due to a reflex response to the demands of temperature

regulation assisted by the mechanical effects of gravity. This may be reversed when circulatory failure is imminent. In the recumbent position with the room at 25° and the relative humidity at 40 per cent, the toe temperature remains considerably lower than that of the finger or forehead (70). The blood flow of reactive hyperemia in the forearm and leg repay the theoretical oxygen debt; this is not the case in the hand, where blood flow is determined more by the demands for heat loss (36). An experimental study of patients with cold hands or with Raynaud's syndrome (64) has shown that emotional stress may be accompanied by enormous reductions in skin temperature in the fingers (up to 13.5°C.). Reactions to emotion and to cold give greater changes in skin temperature in schizophrenic patients than in normal subjects (47, 79).

As demonstrated by changes in skin temperature, vasoconstriction produced by epinephrine in the foot pad of a cat is exaggerated after sympathectomy (67). Dilatation of the skin vessels to warmth is partly dependent on an active vasodilatation through sympathetic nerve channels (51). The rise in skin temperature induced in the arms by heating the legs is greater in a normal arm than in one subjected to sympathetic ganglionectomy. Dilatation is not due to mere removal of vasoconstrictor tone. Dilatation of vessels can produce an increased flow of lymph; such an increase is seen in the cervical lymphatics of dogs subjected to hyperthermia at body temperatures of 38.3° to 41.1°C. A much greater increase develops at temperatures of 41.9° to 43.5°, which is coincident with commencement of circulatory collapse with its concomitant rise in venous pressure and damage to the capillary endothelium by anoxemia (63).

CHEMICAL REGULATION

A careful study of the heat exchange and heat production in both normal males and females in a calorimeter has been made by Hardy & DuBois (90). The data on heat production are surprising. For males lying nude in a temperature of 29° to 31°C. heat loss equals heat production. Below this range heat loss is greater than heat production and the body loses heat. Ultimately increased heat production should be induced. At temperatures above 31° the body gains heat and ultimately this should result in increased metabolism. The heat production in females at the lower temperature is equal to that of the males. On the other hand, there is a

marked reduction in metabolism at the higher environmental temperatures, so that, at temperatures of 30° to 32°, the metabolism is 14 to 20 per cent lower than that of the men. The response of a reduced metabolism on exposure to warmth in addition to that of an increased metabolism on exposure to cold gives the women a considerable advantage. The layer of insulating fat is also usually thicker than that of the men. In the cold they can use this insulation and so attain surface temperatures averaging 1°C. lower, thus conserving heat. The reduction in metabolism at higher temperatures reduces the need for heat dissipation, so that they sweat less than the men. Blood flow through the fat is adequate to remove its insulating power, so that surface temperatures in the warmth are raised to a level averaging 1.7° above that of the men. A greater proportion of the regulation can therefore be accomplished by control of radiation and convection losses. Coupled with the greater adaptability of women's clothing to high environmental temperatures, this series of reactions may be considered as grossly unfair to "organized males."

The data imply that the basal levels of metabolism for men and women on a surface area basis are not different but identical, if the temperature is low but not low enough to induce a shivering reaction. At higher comfort levels the metabolism of women is less owing to the reaction described. The consequent differences have determined the accepted basal metabolic levels.

The development of chemical regulation of temperature in birds is described. With newly hatched birds the metabolism decreases with fall in temperature; after the ninth day it rises (91). Evidence of slow adaptations of metabolism to the external environment will be discussed separately.

SLOW ADAPTIONS TO EXTERNAL TEMPERATURE

The contrasts between winter and summer frogs have long been recognized. There is evidence that the differences depend on endocrine adjustments. The lethal temperature for the intact frog heart is 26° to 27°C. in the winter, and 35° in the fall (103). Injections of whole pituitary in the winter, sufficient to induce ovulation in female frogs, raises the winter level as high as 38°. The pituitary effect is attributed to an influence on the thyroid, since it has been shown by others that thyroxin raises the lethal temperature for the heart. The water transport through frog skin in response to pitui-

trin is greater in summer than in winter (83). The depression of the response in winter does not appear to be due to excessive action of the anterior lobe of the pituitary (82). The possibility of interactions between the thyroid and the posterior lobe of the pituitary in water balance is supported by the exaggeration of diabetes insipidus by thyroid extract (88).

Many of the chemical regulations to temperature changes appear to belong to the category of slow adaptations. Presumably variations in thyroid activity are concerned and are unlikely to be produced rapidly. In mammals thyroid activity appears to be reduced in summer, in contrast to changes in the frog. Heat production in the fasting rat at environmental temperatures from 7.5° to 35°C. has been measured, the basal metabolic rate being found constant from 30° to 33°C. (104). The basal metabolic rate of rats maintained in a room at 7.8° to 12.2° for periods up to ninety days increases 11 to 16 per cent, the maximum being reached in fifteen to thirty days (100). Utilizing the cell-height index, an increased activity of thyroid on exposure of rats to cold has been found. There was an immediate response in the first three days and a later secondary hypertrophy between the 14th and 46th days. This later change is attributed to iodine deficiency (102). Utilizing a similar index, Uotila (105) studied the effects of hypophysectomy and sympathectomy. The thyroid atrophy which follows hypophysectomy could not be prevented by cold stimulation; this fact inferentially points to the anterior lobe as a mediator. Thyroidectomized rats kept in a cold room show little increase in metabolism compared to the normal (98). Administration of thyroxin to guinea pigs produces less effect on metabolism in summer than in winter. If the thyroid be excised, the winter sensitivity is regained. The presence of a thyroid product which decreases sensitivity to thyroxin is assumed (94). The relation of oxygen consumption to environmental temperature during the growth of hypophysectomized dwarf mice is described (81).

The basal metabolic rate in man may be modified by racial differences but none the less is commonly low in the warmer climates, except when complicated by the effects of altitude. The basal metabolic rate in New Orleans is found to be about 10 per cent below normal (87). A similar subnormality is found in Bombay (95). The metabolism of Eskimos is said to be 18 per cent above normal standards (85). In Batavia the metabolism of natives is approxi-

mately normal, while that of Europeans born in Batavia falls little below normal standards (97). On the other hand, Europeans of less than three months residence have their metabolism reduced by an average of 7.9 per cent. Altitude is also a factor, since natives living at altitudes of 1,000 meters show a metabolic rate 9.4 per cent above normal. A similar effect of altitude is indicated in the high metabolic rates recorded in Guatemala in spite of its tropical location (86). The basal metabolic rate in Calcutta was found to be somewhat below American standards in the monsoon period and was decreased still further if the subjects were examined in an air-conditioned room at 24° to 27°C. with 50 per cent humidity (96).

A comparable lowering of the basal metabolism of a subject acclimatized to heat, when exposed to a cool room, has been observed in our laboratories. It lasts for several days. It is associated with a lowering of mean body temperature dependent mainly on a fall in surface temperature. A possible explanation is a rise in metabolism in warmth due to an increase in mean body temperature, later compensated by a decreased basal rate through endocrine adjustments, possibly involving the thyroid. On exposure to cold the lowered body temperature, if inadequate to induce shivering, decreases metabolism still further until the compensatory reactions are evoked (84).

On return to a normal environmental temperature (21.1° to 24.6°C.) after prolonged exposure to warmth (32.0°), the mode of heat loss is modified, presumably due to a series of adjustments with different rates of development. The caloric value of the food consumed is greater, though basal metabolism is at first subnormal. No data are available as to the total metabolism of the day (84). Vasoconstriction and reduction in the size of superficial veins reach their maxima slowly; the blood flow through the fingers attains its minimum only after several days (101). Maximal vasoconstriction may be dependent on a simultaneous reduction in blood volume (80). During this period of adjustment, evaporative loss of heat is subnormal and appears no longer to retain its normal ratio to the metabolic level. Under certain conditions estimations of basal metabolism from the water loss may be erroneous (84). In some subjects the reduced water loss was associated with an increased colloidal osmotic pressure. Whether capillary pressure is lowered is uncertain, but there are changes in arterial blood pressure. Whatever may be the initial response in blood pressure,

during the first few days of exposure to cool conditions blood pressure levels are low and then slowly return to normal. There is a marked diuresis in the cold, delayed in onset, and lasting several days. This diuresis does not appear to be due to a rise in blood pressure; interactions of endocrine secretions, such as pituitrin or thyroxin, may be involved. Any such hypothesis, however, depends entirely on generalizations and does not rest on direct evidence.

On exposure to warmth there is an initial slight inefficiency in heat loss resulting in a rise in deep body temperature, which after a few days returns to normal. Basal oxygen consumption is reduced at this later stage. There is a gradual development of peripheral dilatation and rate of blood flow, which is associated with an increase in blood volume. Large changes in blood volume were reported during the phase of adjustment. Much smaller increases in blood volume are described after adaptation to a subtropical climate by Forbes *et. al.* (89). They believe that the discrepancy may possibly be due to the methods employed. However, this is unlikely, as unpublished data by the reviewers show parallel changes in blood volume as estimated simultaneously by carbon monoxide and T1824. In these experiments, however, the volume changes were smaller than those previously described by them. Some unknown factor may also be involved. In warmth the strain on the circulation imposed by standing with a consequent reduction in cardiac output is at first exaggerated, later reduced. After adaptation cardiac output in the standing posture may almost equal that observed with the subject lying down. Blood pressures are initially raised and later fall to normal or subnormal values. Cardiovascular fitness tests are complicated by these reactions. The mode of heat loss varies. At first evaporative losses are increased, but as adjustments are made, a larger proportion of the total is lost by radiation and convection (84, 101).

Studies by Kuno (92) demonstrate that sweat glands are more numerous in subjects born in a tropical climate. Residence in such a climate after the age of two years seems not to affect the number of these glands. Subjects with numerous sweat glands apparently use the moisture more efficiently since their total loss of water from the skin under stress is less. It has also been shown that negroes in Mississippi working on a treadmill attain heat balance more efficiently than white subjects belonging to the same locality (99).

Chloride and water loss through the skin is modified by adaptation to a tropical climate (93).

HYPERTHERMIA AND HYPOTHERMIA

A considerable literature has accumulated through the increasing treatment of clinical conditions by hyper- or hypothermia. Experiments on dogs have demonstrated dilution of the blood with increased blood volume as the result of diathermic treatment yielding additional heat equal to the basal metabolic rate. With greater heat, water loss and blood concentration results (106). In man, with pronounced sweating, blood concentration is the rule.

Exposure to cold has had less experimental investigation. Rectal temperature in rabbits may be reduced to 16°C. before death occurs. At this low temperature respiration ceases but the heart beat may continue at a rate of one to two per minute for an hour or more after cessation of respiration (109). Many interesting observations have been made by Smith & Fay and their associates (107, 108) on patients maintained with rectal temperatures of 24° to 32°C. (75° to 90°F.).

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DEPARTMENTS OF PHYSIOLOGY

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ENERGY METABOLISM

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The following review on the metabolism of energy covers the literature in part from October 1939 to September 1940, inclusive. The review is limited to intact warm-blooded animals and does not consider tissues, organs, invertebrates, or cold-blooded animals. Oxygen absorption values have been used mainly as a measure of the heat production, for relatively few articles on direct calorimetry have appeared during the year.

Basal metabolism of animals.—With rats 98 to 120 days old a comparison has been made of the sum of the metabolisms of twenty of the individual tissues and organs of each animal and the total basal metabolism of the intact animal (18). The ranges of oxygen consumption were from 21.3 to 33.1 liters per kg. of body weight per day. The mean value for summated tissue respiration was 110.08 ml. of oxygen per 150 gm. of rat per hr. or about 66 per cent of the mean basal metabolic rate (B. M. R.). When 25 per cent was deducted from the B. M. R. for minimal functional activity, the summation of tissue metabolism was 89 per cent. The greatest contributions to B. M. R. were skeletal muscle, liver, integument, and alimentary canal, in that order.

Study (29) of the metabolism of guinea pigs, rats, and mice has shown that the region of thermal neutrality for the guinea pig is 30° to 31°, for the rat 28° to 29°, and for the mouse 30° to 33°C. In the order of the species named, the metabolisms were 590, 680, and 740 kilocal. per sq. m. per 24 hr., when the factors used to calculate body surface by the Meeh formula were 9.0, 9.0, and 9.1, respectively. The order of these animals with relation to their basal metabolism is different from that in a previous extensive series of studies on different animals (3), in which it was found that the heavier the animal the greater was the heat production per unit of surface area.

In a study (44) on 74 adult rabbits that ranged in weight from 1 to 7 kg., the zone of thermal neutrality was found to be 28° to 32°C. The total heat production per 24 hr. ranged from 60 to 335 kilocal. and the heat production per sq. m. of body surface

($0.001 W^{2/3}$) per 24 hr., from 534 to 914 kilocal., being in general higher the greater the weight. The males tended to have a slightly higher metabolism than the females. The values per square meter were lower than those previously recorded in the literature, probably due to more rigid adherence to basal conditions of measurement. The total heat production of these rabbits was slightly below the average metabolism of other warm-blooded animals of the same body weight, except the marmot. In another series of observations on 72 adult rabbits, 2.0 to 2.5 kg. in weight, the rabbits were not subjected to fasting as the author considered it difficult to empty the digestive tract (24). The rabbits were measured at 15° to 25°C. Surface area was calculated by the Meeh formula with a constant of $K=12.9$. The values for the basal (?) metabolism fall into two groups. One group (55 per cent of the total number) had a basal (?) metabolism of 36 to 38 kilocal. per sq. m. per hr. and a smaller group, 42.5 kilocal.

From 68 simultaneous measurements of the insensible loss and heat production of twelve adult rabbits at 28° to 29°C., in the fasting condition, it was found that between the limits of 1.5 and 5.0 gm. of insensible loss per hour the relationship between heat production and insensible loss was a linear function and could be expressed by the equation: $H=1.45L+2.32$, in which H equals kilocal. per hr. and L , gm. of insensible loss per hr. (47). The standard deviation of the percentage differences between measured heat production and heat thus calculated was ± 8.9 . The percentage of heat lost by evaporation of water averaged 24.6.

The basal metabolism of eight wild cottontail rabbits (*Lepus sylvaticus*), weighing from 0.87 to 1.22 kg., averaged (at 28° to 30°C.) 64.9 total kilocal. per 24 hr. and 652 kilocal. per sq. m. ($S=0.001 W^{2/3}$). This is 6 per cent higher than that of the domestic rabbit of the same average weight. At 16°C. the metabolism was 49 per cent above the basal level (46).

A preliminary paper on the metabolism of the porpoise (*Tursiops truncatus*) reports for animals weighing 180 kg., at rest, an oxygen consumption of about one liter per minute (33).

Fasting.—The nitrogenous metabolism and the respiratory metabolism were studied on eleven adult female dogs (9 to 22 kg.), and direct heat measurements were made in a calorimeter (13). The fast was terminated after the loss in body weight had reached about 50 per cent, which required from thirty-eight to seventy days.

Most of the dogs were then given 50 gm. of glucose, and the total metabolism was measured for the second, third, and fourth hours thereafter. With seven of the dogs, when the loss in body weight was about 30 per cent, approximately 15 per cent of the total heat production was derived from protein and the balance mainly from fat. When the weight loss was about 50 per cent (fourth phase of fasting), from 60 to 100 per cent of the heat production could be attributed to oxidation of protein. This group was called the "high nitrogen" group. A group of four dogs did not show a premortal rise in protein catabolism, as only 14 to 22 per cent of the heat came from protein during the fourth phase. This was called the "low nitrogen" group. There was no consistent difference in the two groups with respect to basal metabolism in the intermediate phase or in the fourth phase of fasting. This similarity in basal heat production and the fact that three of the four dogs in the low nitrogen group fasted for a longer time than those in the high nitrogen group suggest that there was a greater store of body fat in the low nitrogen group. The specific dynamic action of the glucose was about 2 kilocal. per hr., in four of the six dogs, or about 50 per cent of that of the well-fed animal. The results indicate a very efficient utilization of body protein when it is mobilized to meet the energy requirements of a fat-depleted organism.

Food supply and food substances.—The effect of the percentage of protein in the diet was studied in a seventy-day balance experiment with mature albino rats of about 400 gm. in weight (20). The protein constituted 10, 25, and 45 per cent of the food supplied, which had a total energy value of 4,220 kilocal. for the period. The total heat production in the three groups, respectively, was 3,505, 3,456, and 3,402 kilocal. The rats remained in approximate equilibrium in respect to live weight and nitrogen content of the body. The metabolizable energy diminished in order of increasing protein content of the diet, principally because the increase in urinary energy more than counterbalanced the decrease in feces energy. The authors suggest, in view of this work, that it would be ineffective to diminish the proportion of protein to nonprotein food in the diet in any attempt to diminish heat production during hot weather.

The metabolism of rats on a calcium-deficient diet was compared with that of control rats in paired feeding experiments in which the pair mates were kept equal in weight by restricting the

food intake of the calcium-supplied controls (38). In three successive series of observations (when the rats were 70, 91 and 97 days old) the heat production of the calcium-deficient rats averaged 116, 122, and 132 per cent of the heat production of the corresponding controls, the difference between the two groups increasing as the deficiency became more severe. The "total efficiency" of utilization of food energy was decreased by calcium deficiency, not only by a lowering of the food intake and a higher basal metabolism but, in addition, by a lower "partial efficiency."

Thirteen pairs of litter mates of rats that weighed 35 to 40 gm. were placed on Sherman-Chase's vitamin-B₁-free diet (40). The control animals were given 25 or 50 μ g. of vitamin B₁ daily. A significant reduction of 13.6 per cent in the oxygen absorption was observed during avitaminosis.

In continuation of earlier work (41) on the metabolism of amino acids with reference to respiratory exchange and heat production, observations have been made with rats fed tyrosine, aspartic acid, and asparagine as supplements to a mixed maintenance ration (42). The plan and methods used were the same as in the previous study. Following the basal diet periods, six rats received in addition to the basal ration 1.288 gm. of *L*-tyrosine, five received 2 gm. of *L*-aspartic acid, and five 2 gm. of *L*-asparagine daily for eight days. Urine and feces were collected during the last five days. The metabolizable energy values of the substances were 59.2 per cent (tyrosine), 71.8 per cent (aspartic acid), and 60.6 per cent (asparagine) of the gross energy of the amino acids. The quantities metabolized, expressed in kilocalories per gram of urinary nitrogen were 45.7 (tyrosine), 20.6 (aspartic acid), and 10.0 (asparagine), and the kilocalories per liter of oxygen were 4.72, 4.40, and 4.30 for the respective substances.

Activity.—Observations on the metabolism of Light Sussex cockerels show that there is a rhythm in the heat production during fasting, as values obtained in the morning were about 9 per cent higher than those obtained in the night (14). The metabolism in the standing position was 40 to 45 per cent higher than that in the sitting position. At the moment of rising the heat output was occasionally trebled but averaged about 100 per cent above that during sitting. In general, somewhat more than twelve out of each twenty-four hours are spent standing, when the birds are kept in cages.

Body build.—A systematic study has been made of the interrelation of body build and oxygen consumption at rest and during exercise with thirty-four adult males ranging in age from twenty to thirty-eight years (54). The more "linear" persons and those with relatively short upper and lower extremities, long torsos, flat chest conditions, and narrow hips relative to breadth of shoulders, had a higher oxygen absorption in the resting condition than "lateral" individuals, irrespective of whether the values were related to body weight or body surface. In moderate exercise smaller oxygen absorption per kilogram of body weight and greater mechanical efficiency were shown by persons of the "lateral" type than by those of the "linear" type. Greater capacity per kilogram of body weight for supplying oxygen to the tissues in exhausting work on a treadmill was shown by individuals who were more "linear" than "lateral" and who possessed short upper and lower extremities and long torsos. There seemed also to be a racial variation in relation to oxygen consumption, as expressed by the craniofacial indices.

Size.—In a study on the relation of oxygen consumption and environmental temperature to the growth of dwarf mice, 87 determinations were made at 33°C. on dwarf mice at various ages and compared with a small number of measurements on young normal mice (9). A marked relationship between rate of growth and basal oxygen absorption was noted. A 16 per cent average decrease in weight of dwarf mice at the critical period (which begins about the seventeenth day of age) was paralleled by a 17 per cent drop in oxygen absorption. A restoration to the pre-critical level occurred when the body weight increased. The youngest dwarf and normal mice had the same rates of oxygen absorption per unit of surface, but, whereas the rate of the normal mouse doubled during the second week that of the dwarf stayed low. In older animals the metabolism of the dwarf was 40 per cent below that of the normal mouse.

A statistical analysis of data on adult rabbits (45), obtained for the purpose of finding the best method of predicting the basal metabolism of the domestic rabbit, demonstrated that for each kg. increase in weight above 1 kg. up to 7 kg. the total heat production increased at a constant rate and could be predicted by the equation: $h_{\text{total}} = 40W + 20$, in which h_{total} represents kilocal. per 24 hr. and W , the weight in kg.

The total metabolism of eleven healthy premature infants averaged 68 kilocal. per kg. per 24 hr., that is, 18 per cent above the normal basal level of 58 kilocal. (25). Diets that supplied approximately 120 kilocal. per kg. per 24 hr. permitted storage of 34 kilocal. A diet of this energy content seems to be sufficient for healthy premature infants more than two weeks old. A distinctly different finding is reported by another worker in the same field, who came to the conclusion from metabolic studies on premature infants that their energy requirements were below 40 kilocal. per kg. and that overfeeding was more general than underfeeding (39). The author considers that the problem of basal metabolism and energy exchange in premature infants requires much new investigation.

In 72 obese children the total basal metabolism was higher, on the average, than that of normal children of comparable height and age, and calculation of the B. M. R. according to different standards gave widely divergent results, none of which can be considered correct (10). When compared with Talbot's height and weight standards, a correlation coefficient of about $+0.9$ was found between the percentage weight excess and the B. M. R. difference. No relation was found between the B. M. R. and the serum cholesterol content. Study of the relationship between basal metabolism and creatinine output has been extended to include an investigation with obese children, twenty-one girls and five boys, aged five years eight months to eleven years eleven months, averaging 3.3 inches taller than the average for their age (61). The data indicate that the B. M. R. of the protoplasmic mass is moderately elevated in adipose children. When referred to the creatinine standard, the average B. M. R. of these obese children was $+14.1$ per cent, when referred to the weight standard -4.1 per cent, and when referred to the height standard $+21.7$ per cent. These findings, taken together, seem to indicate that the B. M. R. in obesity is either normal or moderately elevated. This is in line with the findings of previous workers on the effect of obesity on the metabolism of animals (4, 5).

Athletics.—In a study on circulation in athletes, the B. M. R. of fourteen college athletes was determined (60). Eleven healthy males served as controls. The athletes had a B. M. R. of from -24 to $+5$ per cent, with an average of -8 per cent, and the controls of from -19 to $+4$ per cent, with an average of -7.6 per cent.

In general, the measurements on circulation and related factors showed no noticeable differences between the two groups.

Race.—The B. M. R. of sixty normal Indian men, measured in Bombay, averaged -8.0 per cent from the Aub-Du Bois, -5.3 per cent from the Harris-Benedict, and -9.7 per cent from the Boothby-Berkson-Dunn standard (59). The twenty-four hour urinary nitrogen excretion in the sixty subjects seldom exceeded 7 gm. The low B. M. R. of the normal Indian subjects is ascribed to the very low protein content of the usual Indian dietary and not to racial or climatic factors. The B. M. R. of 154 Malayan school boys of eight to fourteen years of age was measured and compared with various prediction standards, which differ among themselves (51). The values for the Malayan boys fell below two of the standards by about 6 to 7 per cent but exceeded the other two standards by the same amount. These comparisons confirm an earlier conclusion based on work on Malayan adults, that there is no reason why the League of Nations standards for energy requirements should not apply also to the tropics.

A preliminary statement regarding a study of the basal metabolism of Chinese states that during the year 1939 observations totaling 255 were made on 159 patients and eleven normals (17). All patients were in a nonfebrile state, but fifteen were suspected clinically to have thyroid involvement and 144 were convalescent from other diseases. Apart from the thyroid cases, there was no evidence of marked variation from normal standards. With reference to the Mayo standard, the average B. M. R. of the women was $+0.6$ per cent and that of the men -2.8 per cent. Thirty-three nurses showed an average of -1.7 per cent. The results indicate that the racial factor is not of enough significance to warrant a special standard for Chinese. Eighteen Chinese girls and twenty-two Chinese boys, varying from ten to seventeen years of age, born and raised in the Peiping area, were subjects of basal metabolism measurements (43). The results for the girls were somewhat higher than those obtained in an earlier study on American-born Chinese girls (7) but much lower than those obtained in another study on American-born Chinese girls (67). The values for boys were lower than those in the latter study, except when calculated on the basis of kilocal. per cm. of height. The heat production per sq. m. per 24 hr. tended to decrease with increasing age and averaged 998 kilocal. for the girls and 1,079 kilocal. for the boys.

Evidence is accumulating that the deviations in the basal metabolic rates of racial groups from the normal Caucasian standards are not ascribable primarily to actual racial differences. The metabolisms of races are usually affected by so many other factors, such as climate and diet, that it is difficult to arrange a study in such a way that the factor of race is the only one exerting an influence.

Disease.—Five patients with hopeless metastatic carcinoma were subjected to cold treatment, and their basal metabolisms were measured before and during the treatment (66). The metabolism was lowered in every case by the cold. The B. M. R. before treatment varied from -9 to $+45$ per cent and during treatment (after fifteen to thirty-six hours of body temperature under 95°F.) from -37 to $+28$ per cent. Two of the patients showed little change in basal metabolism, although their rectal temperatures during the treatment were 87.4° and 88.2°F. Over a period of ten months, thirty-three patients in the terminal stages of cancer were subjected to 75 individual inductions of reduced body temperature ranging from 74° to 90°F. (58). The basal metabolism was apparently reduced from 20 to 25 per cent.

In children with tubercular bone lesions the basal metabolism was normal, unless the bone lesions were accompanied by active pulmonary lesion (63). A series of 114 basal metabolism tests on 66 afebrile children with the adult type of pulmonary tuberculosis indicated that pneumothorax therapy lowered the basal metabolism (64). The longer the pneumothorax was maintained, the lower was the metabolism. Studies were also made on seventy afebrile children with tuberculous pleurisy (65). Forty of them had active primary tuberculosis associated with tuberculous effusion, and the majority of these had an increased B. M. R. ranging from $+14$ to $+53$ per cent, with an average of $+25$ per cent.

Glands of internal secretion and hormones.—With rats it was found that preparations of the pituitary gland, rich in the melanophore hormone, varied considerably in their effects on oxygen consumption, but that there was an average depression of metabolism in thyroidectomized rats of 23.2 per cent and in hypophysectomized rats of 34.6 per cent (62). When ovariectomized and thyroidectomized rats were made hyperthyroid by feeding thyroid, and stilbestrol was given to some of them immediately after cessation of thyroid feeding, the hypothyroid basal metabolic level was

reached in half the time by the rats that received stilbestrol (56).

With chloralose-anesthetized cats, intravenous injection of adrenalin at rates from 0.00025 to 0.01000 mg. per kg. per min. increased the oxygen consumption 6 to 8 per cent during the five-min. period of injection (26). At rates below 0.007 mg. per kg. per min., the oxygen absorption continued to increase to a maximum five to ten min. after the injection was over. The maximum calorogenic effect (+16 per cent) occurred at this time, after injection at the rate of 0.004 mg. per min. The intramuscular administration of 1 mg. of adrenalin chloride in 0.1 per cent solution to four normal fasting men resulted in an increase of 13 per cent in the metabolism in the $2\frac{1}{2}$ hours after injection (16).

The basal oxygen consumption rose, on the average, 45.7 per cent when three daily doses of thyroxin (2 mg.) were given to cats, but only 25.4 per cent when, in addition to thyroxin, three daily doses of carotene (39,000 U.S.P. units) were given (57).

When rabbits were thyroidectomized, there was a sharp rise in serum cholesterol content, which varied from 81 to 340 per cent above the level before operation (19). The serum cholesterol content became stabilized after about twelve weeks at a value 21 to 112 per cent above the base level. The basal metabolism did not fluctuate so much but gradually decreased during six weeks to nearly 40 per cent below the preoperative level. The thyroidectomized rabbits were much more sensitive than normal rabbits to a single dose of thyroxin, as shown by the changes in basal metabolism, serum cholesterol content, and creatine excretion.

Several studies have been reported on the effect of the thyrotrophic hormone on the basal metabolism of the rabbit (48, 49, 50). The daily injection of 100 to 200 units into an adult rabbit weighing 2 to 3 kg. regularly caused a definite increase in basal metabolism. This increased from the second day on and reached a first maximum of +20 to +40 per cent. The curve frequently presented a second maximum, less elevated, towards the twelfth day. Then it fell rather abruptly below normal. The total duration of the stimulus to hypermetabolism was twelve to sixteen days. If the rabbit with lowered metabolism was left at rest for several weeks, its metabolism gradually attained a normal value. The animal seemed to have returned to its normal condition, but a new series of injections resulted without difficulty in a fall of metabolism. Three normal rabbits received the thyrotrophic hormone for pe-

riods of twenty to twenty-five days, and the basal metabolism increased 26 per cent on the fifth day, 20 per cent on the sixth day, and 30 per cent on the fourth day, respectively. After the twelfth to the fourteenth day it fell below normal and reached values of -21, -30, and -16 per cent. Three rabbits that had previously shown a period of inversion of the effect of this substance were allowed to live without the hormone for from four to six months and were then again given the hormone. The oxygen consumption of these rabbits increased 60, 29, and 36 per cent. The rabbit showing the 60 per cent increase, which had received 166 units per day, died shortly afterwards of acute hyperthyroidism. The other two rabbits, receiving 100 units per day for twenty-one to twenty-seven days, showed a decrease in metabolism following the increase. The author concludes that the period of inversion of the effect of this hormone is transitory and results from functional modifications which are spontaneously reversible.

Drugs.—After total or partial removal of the thyroid gland of the young guinea pig, the course of the basal metabolism usually corresponded to the magnitude of the gland that was not removed (32). With total and seven-eighths thyroidectomy the basal metabolism decreased, but not with five-sixths or less. Iodine given to totally or partially thyroidectomized animals immediately after the operation in either large or small doses, resulted in a rise in basal metabolism when the remnant of the thyroid gland was about one-sixth of the whole gland. Iodine had no influence on the basal metabolism of normal guinea pigs.

The intramuscular injection of 0.5 or 1.0 mg. of benzedrine per kg. in fasting dogs resulted within about ten minutes in an increase in oxygen consumption, which in four of five dogs amounted to 21 to 38 per cent (15).

The intramuscular injection of 20 mg. of amphetamine sulphate in 2 per cent solution resulted, with four normal fasting men, in an increase of 7 per cent in the metabolism during the $2\frac{1}{2}$ hours following injection (16).

A study was made of the effect of dinitrophenol on normal and schizophrenic subjects in relation to insensible loss, metabolic rate, and skin and rectal temperatures (21). There were twenty subjects in each group, and the patients were matched for height and weight with the normals, who lived under exactly the same

conditions as the patients. The measurements were conducted at an air temperature of 30°C., a relative humidity of 20 per cent, and an air movement of 5 ft. per min. On one day the subjects were studied under basal conditions. Seventy-two hours later they were given 300 mg. of dinitrophenol by mouth, one hour before going to the laboratory. After administration of dinitrophenol the normals showed an increase in skin temperature up to 2.2°C., a rise in oxygen consumption of 31 per cent, and an increase in evaporation rate of 51 per cent. The patients reacted less to the drug, as the corresponding changes were 1.3°C., 25 per cent, and 28 per cent.

With eighteen men and twenty-one women the metabolism after cigaret smoking increased in 82 per cent of the subjects, decreased in 13 per cent, and showed no change in 5 per cent (30). On the average, it increased 8.9 per cent. More marked changes were noted with women than with men. The maximum effect of one cigaret occurred at once with some subjects and was delayed for forty-five minutes with others. Most of the subjects showed a second slight increase after about forty-five minutes. In general, those who inhaled the most smoke showed the greatest physiological changes. Abstainers and those who smoked only occasionally showed marked changes, but habitual smokers who inhaled little or no smoke showed only moderate effects. Those in a basal metabolic condition experienced greater changes than those not in a basal condition.

Season, climate, and environment.—The spermophile, *Citellus citellus*, L., adapted to temperatures of 18° to 32°C. during the summer, was found to have a rectal temperature varying between 35.7° and 39.1°C. and reacted to variations customary at this season as a perfect homoiotherm (22). Its basal metabolism in the summer was less elevated than that of rats and birds under these conditions and was about 450 kilocal. per sq. m. ($K=10.38$) per 24 hr. The results of a two-year investigation of the seasonal metabolic rhythm of domestic fowls during the pullet year showed that there was a rough parallelism between fasting heat production, heart rate, and egg production (68). There was considerable daily fluctuation in metabolism and heart rate, which was as great as 40 per cent of the highest figures found in one month. A study, in India, of the B. M. R. of a man on 43 days between September 1936 and November 1937 showed no marked effect of season, as most

of the results were within 2 per cent of the average (52). The results in May and June were slightly higher than those in November and December.

The respiratory exchanges of adult house wrens (*Troglodytes aëdon*) and of nestlings from the time of hatching to fifteen days of age were measured at various environmental temperatures with the open-circuit Haldane apparatus (36). At thermal neutrality (37.8°C.) the heat production increased with age, at least until temperature regulation was well established, that is, from 264 kilocal. per sq. m. per day at hatching to about 1,000 kilocal. in twelve- to fifteen-day-old birds. The temperature of 37.8°C. represented a critical point above which regulation of body temperature was effected primarily by increasing heat loss rather than by further decreasing heat production.

An investigation (34) of the effect of temperature on oxygen consumption was made with the woodmouse (*Apodemus silvaticus*, L.) and the yellow-necked mouse (*A. flavicollis*, Melch.). At 30° and 35°C. the oxygen absorption per unit of body weight was greater in yellow-necked mice than in wood mice. At 0° to 25°C. the opposite was true. In both species the oxygen absorption decreased with increase in temperature from 10° to 35°C.

Six dogs (5 to 9 kg.) had their basal metabolisms measured individually at 29.5°C., in a closed-circuit respiration apparatus (55). Then they were subjected to a temperature of 35.5°C. and a humidity of 50 per cent, and measurements were continued for seven half-hour periods. During this time the oxygen consumption showed a progressive climb to values 50 to 100 per cent above the basal values. The water loss during panting averaged 50 mg. per kg. per hr., which was eight to ten times the basal value. The heat production of a non-heat-regulating dog (pontile animal), after a 24-hour fast, was studied at rectal temperatures from 30.5° to 38° C. (11). When the heat production was plotted against rectal temperature, there was a straight-line increase of 12 per cent in the heat production for each degree rise in rectal temperature. This percentage of increase appears to be of about the same order of magnitude as that of a febrile human subject. The metabolism of this dog was much higher than that of cold-blooded animals at the same temperature.

A systematic study of various hibernating and non-hibernating animals has been made with respect to changes in basal metabolism

as related to environmental temperature (35). The measurements were made either by the gravimetric method of Haldane with chambers adapted to the sizes of the animals or by the method of confinement, in which the composition of the confined atmosphere was analyzed at the beginning and the end of each period. Pigeons (*Columba*), which lived in the laboratory at temperatures varying from 15° to 25°C. in the course of the year, had an average heat production per sq. m. per 24 hr. of 743 kilocal. at 28.4°, 737 kilocal. at 20.8°, and 1922 kilocal. at -12.8°C. The heat lost by vaporization of water varied from 7.6 per cent at 2.5° to 43.3 per cent at 36.5°C. Marmots (*Arctomys marmota*) lived in the laboratory at 10° to 15°C. and were measured during September and October. At thermal neutrality (29.6° C.) they produced 392 kilocal. per sq. m. per 24 hr. This compares favorably with an earlier finding at 28°C. (6) of 410 kilocal. At -8.2°C. they produced 1,339 kilocal. Hedgehogs (*Erinaceus europaeus*) at 29.1°C. had an average heat production of 740 kilocal. and at -12.7°C., 2,225 kilocal. per sq. m. per 24 hr. Many of the measurements at the lower temperatures were complicated by activity. Hamsters (*Cricetus frumentarius*) lived at 11° to 24°C. and were measured at 28.9°C. in one set of experiments, when they had a heat production of 684 kilocal. per sq. m. per 24 hr. At -7.5°C. they produced 1,815 kilocal. per sq. m. per 24 hr. Spermophiles (*Spermophilus*, *Citellus citellus*) lived at temperatures between 13° and 20°C. At 28.6°C. the heat production was 600 kilocal. and at 3.8°C., 1,539 kilocal. per sq. m. per 24 hr. The chemical regulation of the spermophile was far from perfect. Dormice (*Myoxus glis*) lived at 11° to 19°C. with the exception of July (22° to 23°C.). At an average temperature of 29.6°C. the heat production averaged 527 kilocal. per sq. m. per 24 hr. and the rectal temperature, 38.4°C. At -13.8°C. the heat production was 2,175 kilocal. per sq. m. per 24 hr. and the rectal temperature, 35.5°C. The lerot (dormouse; *Myoxus arbor*), when adapted to a temperature of 20°C. and after fasting seven hours at 20°C. before each measurement, had a heat production of 696 kilocal. per sq. m. per 24 hr. at 28.3°C. and of 1,893 kilocal. at 1.0°C. When it was adapted to 8°C. and then measured at 27.9°C., the heat production was 908 kilocal.; at 1.2°C. it was 2,232 kilocal. per sq. m. per 24 hr. The muscardin (dormouse; *Muscardinus avellanarius*) lived at 13° to 18°C. and fasted for seven hours at 20°C. before each measurement. Only the values at thermal neutrality were uncom-

plicated by activity. At 27.6°C. the heat production was 832 kilocal. and at -11.3°C. 2,856 kilocal. per sq. m. per 24 hr. The author also includes a discussion of the modifications in the various glands of the different animals in respect to adaptation and season.

The effect on the heat production of changing to an environment of 36.0°C. was studied with rats that had been living at temperatures of 8° to 15°, 14° to 22.5°, and 29° to 32°C. for periods varying from 24 to 115 days (23). The environment of 36°C. is in the zone of thermolysis, that is, in that range of environmental temperatures in which the regulation of body temperature is maintained by loss of heat. The rats were then kept at 36°+ for six weeks. On the first day of measurement at 36°+ the heat production averaged 696 kilocal. per sq. m. per 24 hr. following adaptation at 29° to 32°C., 845 kilocal. following adaptation at 8° to 22.5° C., and 912 kilocal. following adaptation at -2° to +12°C. The author states that the rat, like the bird, produces more heat at a definite temperature lower than thermal neutrality when it is adapted to a cooler thermal environment. When rats were transferred from a low temperature to 36°C., they produced 850 kilocal. per sq. m. per 24 hr. at first but after fifteen days only 640. A rat maintained at 29° to 32°C. for 115 days had a heat production of 726 kilocal. per sq. m. per 24 hr. When it was transferred to 36.6°C., its heat production rose to 951 kilocal. on the third day and decreased to 564 kilocal. in 42 days. Rats that came from 15°C., at which they produced 1,150 kilocal. per sq. m. per 24 hr., produced at the beginning of their stay at 36°C., only 850 kilocal. On the contrary, those that had lived a long time at 29° to 32°C., with a heat production of 600 kilocal. per sq. m. per 24 hr., produced 700 at the start at 36°C. The experiments indicate in general that in the zone of thermolysis the body temperature does not depend solely on the intensity of heat production for the same rat produced 703 kilocal. per sq. m. per 24 hr. (body temperature, 38.2° to 39.6°C.) in one case and 577 (body temperature, 38.0° to 39.8°C.) in another case, according to whether it had been a long or a short time at 36°C.

Measurements of the basal metabolism of men and women of different vocations, in Rio de Janeiro, show that under normal conditions, independent of climate, race, and biological type, the basal metabolic levels of these subjects compared favorably with values reported for people in other countries (1). The conclusion

drawn is that the food regime in Brazil is equivalent to that of the countries of Europe and North America. A comprehensive study of the basal metabolism of human subjects in Rio de Janeiro in the comfort zone and at various other environmental temperatures and of the effects of anthropological and constitutional types is reported in a monograph by the same author (2).

The operative factors of seaside climate were studied from September to November on thirteen boys between seven and fifteen years of age (37). These boys had had extrapulmonary tuberculosis but were convalescent or at least apyrexial during the period of study. Their respiratory exchange was measured while they lay in the open air throughout the day, dressed in night shirts and covered with bed clothing. Control observations were taken indoors and were therefore uninfluenced by sun or wind. The controls agreed with the usual prediction tables for basal metabolism or were slightly above them. The metabolic rate was increased in the open air, both in the sunshine and with exposure to winds of strong cooling power.

To ascertain whether the B. M. R. values obtained on children at Denver, Colorado, were normal in spite of the altitude, a study (31) was made of the B. M. R. of five women and two men at Denver (altitude 5,280 feet) and at Stillwater, Oklahoma (altitude 910 feet), and with four of the subjects also at Eldora, Colorado (altitude 8,720 feet). The subjects remained at each altitude long enough to become acclimatized, as judged by measurements of certain physiological functions. Altitudes up to 8,720 feet had no influence on the B. M. R.

A brief report has been made of a study of temperature in white men and negroes, in which two hours' work on a treadmill was carried on at such a rate that the metabolism was about eight times the basal level in the average man (53). Twenty negro sharecroppers were able to attain a balance between heat loss and heat production after thirty minutes of work and to continue the work with an average rectal temperature of 38.2°C. The rectal temperatures of seven white sharecroppers continued to rise for 105 minutes. The negroes were slightly more efficient in the work, as they required oxygen at the rate of 24.9 cc. per kg. per min. in the first fifteen minutes and 25.6 cc. at the end, whereas the white sharecroppers required 25.9 and 27.3 cc., respectively.

In a study on the effect of air movement on heat losses from the

clothed human body, two men were exposed (at a relative humidity of 40 to 50 per cent) to air movements of 4.6 cm. per sec. at 16.1°, 34.0 cm. at 19.2°, and 264.0 cm. at 22.8°C. (69). The subjects were lightly clothed and in a semi-reclining position. All three curves for metabolism showed a fall of 2.5 to 4.5 kilocal. per sq. m. per hr. during the first two hours, with a subsequent rise which reached or exceeded the original value in two of the three cases. This phenomenon has been previously described (8). The authors agree with the earlier authors that the fall at the start was due to relaxation of the subjects and the increase later to slight restlessness.

An investigation has been made of the slow adaptations of man to altered climatic conditions, with respect to changes in heat exchange (12). Two subjects were studied at a time (in all there were six subjects) while living in air-conditioned rooms at either high (28.6° to 32.6°C.) or moderate (21.1° to 24.6°C.) temperatures. The duration of stay at any one temperature was from one to ten days. One series of experiments was conducted in winter, two in summer, and one in spring. Computations of the daily food intake were made in terms of calories for all experiments, and for two experiments in terms of protein, fat, carbohydrate, and water content. In one set of experiments two subjects lived four days at 32.4° and then four days at 21.1°C. During this time their daily energy intake at 32.4°C. was 1,120 and 1,270 kilocal. per sq. m., respectively, and at 21.1°C., 1,344 and 1,602 kilocal. In other words, these two subjects showed increases in caloric intake of 20 and 26 per cent when living at a moderate temperature following a warm temperature. One of these subjects, who was in both a winter and a summer series, had a caloric intake 16 per cent greater in the hot room in winter than in summer, which may indicate a persistence of "winter appetite." An analysis of the food showed that there was a shift from carbohydrates to fat when the temperature of the room was lowered. The R. Q. of the food was 0.84 and 0.86 for these two subjects in the hot room and 0.82 and 0.83 in the cool room. The basal metabolism of two other subjects measured daily in a similar series of experiments fell sharply in the first few days of cold, the fall amounting to more than 15 per cent. In other series where sensations of cold were marked, there might be instead an initial increase in basal metabolism. The initial decrease in metabolic rate in the cold environment was not far from what would

be expected on the basis of the fall of the average body temperature which was calculated from the skin and rectal temperatures. As the caloric intake, when increased, was not accompanied by a rise but rather by a fall in heat production during the first few days of cold, there was a marked unbalance in the metabolism on these days.

In addition to the studies on three women reported in brief last year (28), the results are now given for four more women who had their respiratory exchange and the partition of their heat elimination measured by a respiration calorimeter while they were in the basal and nude condition, at air temperatures ranging from 22° to about 35°C. (27). The observations lasted approximately 2½ hours. A comparison is made with similar observations on two men. At air temperatures between 23° and 27°C., the average heat production of both sexes was alike. From 22° upwards the heat production of the men was unaltered, but above 27° that of the women fell slowly until at 30°C. it was at the minimum. Thereafter it rose slightly and gradually with the rise in air temperature. The heat loss of the women in the cold zone was about 10 per cent lower than that of the men because of the women's lower skin temperature. In the warm zone the heat loss of most of the women was 14 to 20 per cent lower than that of the men because the women did not sweat so much. The men and women under these limited conditions showed two points of agreement, Newton's Law constant and the internal body temperature. In all the other adjustments to changes in the thermal environment the women had a physiological advantage. The lowering of the metabolism of the women in the warm zone is evidence for the existence of a "chemical regulation" as described by Rubner for animals.

The finding of a variation in the metabolism of women according to the environmental temperature, even though they were unclothed, brings forward the question as to whether more care should not be exercised in the determination of the basal metabolism of women and perhaps of men with respect to the conditions of environmental temperature. Although there is not yet any definite evidence that women in the clothed condition would show an effect of environmental temperature, at the same time this probability can not be entirely ruled out, for it should be recalled that under such conditions the variation in the metabolism of women is, in general, somewhat larger than that of men.

A survey of the literature on energy metabolism, particularly in humans as well as animals, shows that there is increasing study of the effect of environmental conditions, especially with regard to external temperature. Such studies will ultimately be of immense value in the adaptation of man and animals to various climatic conditions, both with respect to ordinary living and with respect to the carrying on of physical activity. These studies also will ultimately prove of value in helping to form a better scientific basis for air conditioning.

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RESPIRATION

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In our opinion, the phases of respiratory physiology in which the developments of the past year have led to the greatest additions to or alterations in our knowledge on the subject are the following: (a) the location and functional integration of the regulating mechanisms in the central nervous system; (b) the interrelationships between nervous and chemical factors in the adjustment of pulmonary ventilation to the needs of the organism; and (c) the abnormalities associated with flight in aircraft, the seriousness of these having been greatly increased by the recent advances in aeronautic engineering. Taking advantage of the preference of the editorial board of this publication for attempts to appraise the present status of a few problems of particular interest rather than simple compendia of the literature, we propose to devote the major part of the allotted space to these three topics and to dismiss others with scantier discussion.

LOCATION AND FUNCTIONAL INTEGRATION OF THE CENTRAL REGULATING SYSTEM

The long sequence of attempts to localize the respiratory center or centers in the central nervous system has culminated in the publication of a series of papers by Gesell and his collaborators (78, 79, 80, 82), Stella (180), and Pitts and co-workers (152, 153). [The older literature is cited by Pitts (153)]. As a result of these studies it now seems certain that the so-called respiratory center of the medulla is not a compact, sharply localized structure, but is scattered bilaterally in the gray matter of the ventral reticular formation of the medulla overlying the upper (cephalic) four fifths of the inferior olive. The location is practically the same in the dog (80) and cat (153). The diffuseness of this "center" had indeed been suspected for some time, but the tradition of the "*noeud vital*" is so strong that Pitts' calculation (153) that it occupies a space of approximately 30c. mm. in the cat may come as something of a surprise. Still more surprising is the recent confirmation by Stella (180) and Pitts (153) of many of the conclusions reached nearly

twenty years earlier by Lumsden (125). The latter had claimed that the medullary center, when cut off from efferent impulses from stations higher in the neuraxis and from afferent impulses carried by the vagi, is no longer capable of rhythmic activity but can only induce a sustained inspiration for which he coined the term "apneusis" (a finding that is fully confirmed by both Stella and Pitts); that in the medullary center there are anatomically as well as functionally separate cell groups for the inspiratory and expiratory muscles [which is confirmed by Pitts *et al.* (153) in the cat, though not by Brookhart (43) in the dog]; that the rhythm of the medullary inspiratory (or apneustic) center is determined by another center in the upper part of the pons, which Lumsden called the "pneumotaxic" center. Stella and Pitts have also confirmed this, though with the important difference that they find the rhythm of the medullary center not to be directly set by the pneumotaxic center in the pons (as Lumsden claimed), since the rhythm remains substantially unaltered after transection of the upper pons as long as the vagi are intact. They believe that rhythmic respiration depends on periodic inhibition of the activity of the medullary inspiratory (apneustic) center by nerve impulses aroused by the inspiratory act; this function can be carried out adequately either by efferent impulses from the pontile pneumotaxic center or by afferent impulses carried by the vagi. As to the location of the pneumotaxic center, both Stella (180) and Pitts (153), using different methods (the former transections, the latter electrolytic lesions) have come to the same conclusion, viz., that it lies bilaterally in the ventral part of the tegmentum of the upper few millimeters of the pons, near the midline on each side.

A reciprocal innervation between the inspiratory and expiratory neurons in the medulla, already demonstrated by Bronk & Ferguson (42) in experiments on the nerves supplying the external and internal intercostal muscles of the cat, has been substantiated by Pitts (153) and Gesell (78). Pitts used local stimulation applied to various points in the medulla of the cat and found that inspiration was invariably inhibited by stimulation of expiratory spots and conversely; he suggests that the main function of the expiratory neurons is to inhibit the activity of the inspiratory cells, and that nerve impulses having this function (such as those from the pneumotaxic center and those carried by the vagi) act by primarily stimulating the "expiratory center" in the medulla. Brookhart

(43) does not agree that a discrete "expiratory center" exists (in the dog) and believes that the neurons connected to the antagonistic respiratory muscles are scattered throughout the reticular formation. It is not clear at present whether this difference of opinion rests on technical grounds or species differences. Pitts (153) holds that the pneumotaxic center plays a part in the reciprocal innervation between the inspiratory and expiratory neurons. He pictures conduction of part of the discharge from an inspiratory neuron upward to the pontile center whence it is conducted down again to excite the "expiratory center" in the medulla; from the latter the disturbance is transmitted to the "inspiratory center" as an inhibitory influence. Afferent impulses carried by the vagi are also supposed to act primarily by stimulating the "expiratory center." Gesell's (78) conception of this reciprocal innervation apparently is based on studies of action potentials at various points in the inspiratory and expiratory neuromuscular system in dogs. Instead of an interconnection among various "centers," he postulates an excitatory and inhibitory pole in each neuron; collaterals from antagonistic neurons would carry impulses to the latter while those from fellow agonists would be directed to the former.

The spread of impulses throughout the reticular formation was well demonstrated by Pitts (153), who found that a suitable stimulus applied through punctate electrodes at any point within the limits of the "inspiratory center" in the cat produced a maximal inspiration. Corresponding stimulation in the "expiratory center" produced a maximal expiratory effort. Gesell and his collaborators (79, 81, 82) detected identical electrical disturbances (of the "slowly augmenting type") with inspiration in various parts of the reticular formation of the medulla of the dog and also concluded that the excitation wave sweeps over the entire structure. Pitts (153) suggests that inhibitory nerve impulses impinging on the reticular system may act by increasing synaptic resistance; "apneustic" breathing might be due, he suggests, to re-entry and recirculation of an excitation wave over one particular pathway, and impulses from the pontile pneumotaxic center of the vagi might block this by increasing the resistance to its spread; it is possible, he believes, that adverse chemical influences such as anoxia and severe hypercapnia may also act by increasing synaptic resistance in the reticular formation. It is interesting to note that Rosenthal (160) many years ago suggested that resistance to the conduction

of the excitation process might be an important factor in the causation of the rhythm of breathing.

The importance in respiratory control of parts of the neuraxis other than the medulla and pons has been suggested by several recent workers. Barcroft (11) emphasizes the importance of inhibitory impulses from the cerebrum to lower parts of the neuraxis, including the respiratory center; he believes that the development of this inhibitory control is responsible for the quiescence of the fetus *in utero* as it approaches the end of gestation. Bailey & Sweet (10) found that respiration was inhibited in cats and monkeys by rather weak stimulation of the orbital gyrus of the frontal lobe. Langworthy, Hesser & Grimmer (90, 115) concluded, from studies made in patients with lesions involving the pathways from the cortex to the medulla, that impulses from the cortex normally inhibit the activity of the medullary center. Mansfeld & Hámori (133) found that transection of the pons of the dog at the level of the auditory nerve frequently caused respiratory failure and, when the animals did resume breathing, their sensitivity to changes in the carbon dioxide tension of the blood was practically gone. Stella (179) confirmed these findings in the main; he found that application of procaine to the cut surface of the pons did not lead to restoration of breathing, indicating that the apnea was not due to irritation of inhibitory nerve fibers; the response to carbon dioxide inhalation however was not abolished in his animals. Gentle pressure on the chest sometimes led to the resumption of nearly normal respiration; this Stella ascribes to reduction in the inhibitory discharge carried by the vagi from the lungs. The prolonged or permanent apnea consequent on transection of the pons therefore appears to be due to excessive inhibition of the medullary center by impulses from the lungs. Further work along these lines seems desirable. Monnier (138), from experiments in cats in which "decerebration" (performed by ligation of the internal carotid and basilar arteries) led occasionally to more or less prolonged inspiratory apnea, concluded that the higher parts of the neuraxis normally contribute an inhibitory influence upon the respiratory center in the medullary reticular formation. He also (137) applied faradic stimulation to various points in the floor of the fourth ventricle and claimed that stimulation of one part modified the tone ("posture") of the respiratory muscles while stimulation of another changed the frequency and amplitude of the respiratory movements.

The cerebellum has also come under investigation as a factor in respiratory control. Mansfeld & Hámori (133) concluded that this structure is essential to the respiratory effects of chemoreceptor reflexes since intravenous injections of cyanide failed to cause hyperpnea after the cerebellum had been removed from a decerebrate animal. This could not be confirmed by Stella (178). Moruzzi (139) has recently claimed that the cerebellum can exert an inhibitory effect on the medullary centers, but the evidence is not impressive.

Rijlant (157) proposes a scheme of central control of breathing involving two distinct mechanisms—a fundamental center, and a modulating system which impresses a characteristic form on the other. The relation of this system to that outlined by Pitts and Stella is not clear to the reviewers.

INTERRELATIONS OF CHEMICAL AND REFLEX STIMULI IN THE ADJUSTMENTS OF PULMONARY VENTILATION

For many years the physicochemical aspects of respiratory control were dominant, but during the past decade the reflex factor has grown steadily in stature until, in our opinion, the time has come to reverse the traditional attitude and to look upon the changes in the chemical stimulus in the blood occurring under ordinary physiological as well as most pathological conditions as results rather than causes of changes in pulmonary ventilation. The most important recent developments have to do with (a) the identity of the chemical stimulus to the respiratory center, and (b) the parts played by direct central and reflex factors in physiological adjustments of pulmonary ventilation.

The identity of the chemical stimulus to the respiratory center.—This question, seemingly settled by Haldane's acceptance of the hydrogen ion, has been reopened by the outcome of experiments made during the past few years in Krogh's laboratory (47) with the object of securing a better understanding of the physiology of muscular exercise. In a series of experiments that seem to have been above reproach, Nielsen (146) obtained evidence that the chemical stimulus to the center is actually carbon dioxide, acting as such. One of the chief items of evidence was the failure of acidosis (produced by ingestion of ammonium chloride) to produce more than trifling respiratory stimulation in subjects who showed a vastly greater hyperpnea on breathing carbon dioxide though the

acidity of the arterial blood was increased much more in the former case. Nielsen points out that, since all of his observations were made in "steady states," it is not possible to attribute results such as these to failure of the arterial blood to give a true picture of the state of affairs in the respiratory center (as has often been done to reconcile such observations with the requirements of a dominant chemical control), without also concluding that the center is so slow in responding to changes in the arterial blood as to have little or no value in protecting the organism against such changes.

The conclusion that the carbon dioxide molecule and not the hydrogen ion is the chemical stimulus to the respiratory center is accepted by Krogh (114) in a monograph in which the experience and wisdom accumulated during a long series of distinguished contributions to respiratory physiology are set forth. The reviewers are inclined to accept Krogh's judgment. As to the mode of action of the stimulus on the center, Krogh (114) points out that "the general effect of CO_2 is somewhat similar to that of a narcotic, stimulating within certain narrow limits and above that depressing." Its stimulant effects on the respiratory center may be only one special manifestation of the "excitement stage" of narcosis, which appears to be just as constant a phenomenon as the depressant effect of higher concentrations (193).

Gesell (78) has recently proposed an electrochemical conception of respiratory control: through the metabolic activity of the cell an electrical current is generated and part of this, after emergence from the cell, is conducted back through recurrent collateral branches to augment the original activity. Thus is explained the "slowly augmenting type" of electrical activity which has been found to be associated with inspiration in various parts of the neuromuscular apparatus (79, 82). The inspiratory discharge is thought to terminate abruptly because of exhaustion of the cells by their steadily increasing activity. The characteristic pattern of expiratory activity apparently is a "steady state" discharge which may be modified by afferent impulses into a "rapidly augmenting" type. The reviewers do not see how apneustic inspiration is to be explained on the above basis; if we understand Gesell correctly, the abrupt termination of the inspiratory discharge is attributed to fatigue of the nerve cells and recirculation of part of the original excitation wave plays a large part in bringing this about, but that leaves no basis on which to account for a sustained inspiration.

Recirculation of the original wave was indeed postulated by Pitts (153) as an explanation for apneusis, but he proposed that this is possible only when inhibitory nerve impulses no longer reach the inspiratory center during inspiration. Since some of these impulses supposedly originate in the pneumotaxic center and have nothing to do with respiratory movements, they are not excluded by paralyzing the animal with curare, as was done in Gesell's experiments. The "slowly augmenting type" of electrical discharge was observed in both the internal and external intercostal muscles (82), but since similar studies of the nerves supplying these have shown the former to be an expiratory muscle (42) the basis on which this part of Gesell's conception rests does seem to be entirely sound.

The parts played by direct central and by reflex effects in various respiratory adjustments.—The hyperpnea of muscular exercise and of anoxemia are certainly among the most important of respiratory adjustments, and recent developments have indicated that these are both due much more to excitatory reflexes than to an increase in the chemical stimulus. The discrepancy between chemical stimulus and respiratory response under these circumstances has long been realized and has often been attributed to increased excitability of the center to the stimulus. The factor responsible for this increase now can with some certainty be identified as a new or augmented reflex drive in both cases, though the source of the drive is not the same in both.

(a) *The hyperpnea of muscular exercise.*—The conclusion that this is due to reflexes and not to an increase in the chemical stimulus rests on two types of evidence: first, there is not enough increase in the chemical stimulus to account for it and second, a reflex factor capable of explaining it can be shown to be present. Evidence of the first type has been accumulating for a number of years and Nielsen's (146) experiments were only the climax of a series of such studies, most of which showed that the hyperpnea of muscular exercise is far greater than it should be if it is due to an increase in carbon dioxide tension, a decrease in pH, or a decrease in oxygen tension in the arterial blood. In some cases, as in the experiments of Bock *et al.* (34), there was an increase in one of these stimuli (acidity of arterial blood) and even though the other two were decreased it was still possible that the observed (sevenfold) increase in alveolar ventilation might have been due to the observed decrease of pH (0.074). Nielsen's (146) finding that an increased

acidity of this order (pH 0.08) produced by ammonium chloride ingestion caused only a slight increase (0.7 liters per minute) in pulmonary ventilation at best and might cause none at all, makes it improbable that increased acidity *per se*, accompanied as it was by a decrease in arterial carbon dioxide tension, could have been the cause of the hyperpnea in Bock's experiments. In Nielsen's subjects intense hyperpneas were elicited by exercise without increase in any stimulus in the arterial blood. Acidity and carbon dioxide tension diminished steadily and oxygen tension increased as the exercise and hyperpnea became more severe.

In other experiments Nielsen (145) estimated, from the increase in oxygen uptake produced by inhalation of various concentrations of carbon dioxide in air, that the work of the respiratory muscles represents only about 1.3 per cent of the total energy metabolism when the subject is at rest, but as the minute volume of ventilation increases this proportion rises steadily to reach nearly 9 per cent when pulmonary ventilation is maximal. Evidently the accessory inspiratory and the expiratory muscles are less efficient than those involved in quiet breathing. One of the most interesting findings in these experiments was the sharp and unexpected upper limit to the stimulant action of carbon dioxide. Nielsen (145) found that as the carbon dioxide content of the inspired air was increased, pulmonary ventilation increased in proportion until a concentration of about 9 per cent was reached; further increase in the carbon dioxide content then led to a diminution in the respiratory minute volume. The maximum ventilation that could be obtained by carbon dioxide inhalation in these subjects was 50 to 60 l. per min.; the same subjects had no difficulty in breathing 100 to 120 l. per min. while exercising. The observation that the hyperpnea of exercise is greater than any that can be elicited by carbon dioxide inhalation is not original with Nielsen; Barcroft & Margaria (14) had already commented on this fact, and had also noted that the more effective hyperpnea of exercise is much less uncomfortable to the subject than the smaller pulmonary ventilation produced by carbon dioxide inhalation.

Nielsen (146) alludes to the work of Heymans (100) in which the "increased excitability of the center" during anoxemia was shown to be due to reflexes from the carotid and aortic bodies, and notes that this would offer a satisfactory explanation for the increased excitability of the center during anoxemia; the hyperpnea

of exercise could be attributed to irradiation of impulses destined for the exercising muscles into the respiratory center, or to excitatory reflexes from the muscles. Choice between these is afforded by the work of Harrison and his collaborators (96). They also had found that the hyperpnea of exercise is not due to an increase in any identifiable chemical stimulus; they added observations on anesthetized animals in which hyperpnea occurred when a limb was moved passively, even though all vascular communications were interrupted and the leg was connected with the body only by the sciatic nerve; when the latter was cut, movements of the leg no longer affected breathing even though vascular communications were reestablished. They concluded that the hyperpnea of exercise is due to reflexes from the muscles and not to a chemical factor or to irradiation of impulses from corticospinal tracts into the respiratory center. Comparable observations dealing with the hypertension and tachycardia of exercise were made by Alam & Smirk (3) with entirely comparable results; afferent impulses from the muscles are therefore capable of stimulating the respiratory, vasomotor, and cardiovascular centers. The point of origin of these impulses has not as yet been ascertained. Gesell (78) has suggested that the Golgi apparatus is concerned.

The question of dyspnea in disease is discussed at length by Christie (48) and Harrison (96). As encountered in heart disease, the cause appears to be congestion of the pulmonary circulation which reduces the vital capacity and elasticity of the lungs and gives rise to excitatory reflexes. McMichael (129) believes that the dyspnea of heart disease is more closely related to cardiac output than to vital capacity, and is inclined to emphasize circulatory factors, such as cerebral blood flow, in its production. The presence of a nerve system in the heart by which breathing can be stimulated reflexly is indicated by the work of de Waele & van de Velde (188, 189). Jarisch & Richter (105) conclude that the effects of veratrine, which include apnea, decreased vasomotor activity, and increased cardiac inhibition in the intact animal, are due to reflexes aroused by the drug in the myocardium but these effects, being inhibitory to the respiratory and vasomotor centers, are entirely different from those mentioned above. The dyspnea of silicosis is attributed by Cole & Cole (49) to obliteration by the foreign particles of considerable portions of the alveolar capillaries; this would imply that the responsible factor is an increase in the

chemical stimulus in the arterial blood, which disturbance should be amenable to direct demonstration.

(b) *The hyperpnea of anoxemia (chemoreceptor reflexes).*—This is now known to be due to reflexes from the chemoreceptors in the carotid and aortic bodies; the literature on this subject has recently been reviewed from several viewpoints (77, 100, 165) including the historical (164) and will not be recapitulated here. In the first volume of this series Gesell (77) questioned the propriety of attributing all of the anoxemic response to reflexes, but recently (81) he has come to agree that it is essentially a reflex phenomenon at least in the anesthetized animal. We know of no compelling evidence that the situation is any different in the absence of anesthesia; the evidence cited to the contrary is open to criticism according to Schmidt & Comroe (165) either because the denervation (particularly of the aorta) may have been incomplete or because the test of chemoreceptor function was not a fair one.

Reflexes from the carotid and aortic bodies are known also to be aroused by increase in the carbon dioxide tension or acidity of the blood as well as by a large number of drugs (100, 165), but their importance to the regulation of respiration is still under debate. We have recently (165) reviewed the available evidence and concluded that, as far as carbon dioxide is concerned, the center is much more sensitive than the chemoreceptors, acts just as rapidly, and produces a much greater hyperpnea for a given increase in carbon dioxide tension. The only respect in which the chemoreceptors seemed superior to the center with regard to carbon dioxide is their much greater resistance to adverse circumstances. Under certain experimental conditions (particularly deep anesthesia by chloralose) the reflexes become more important, but this seemed to us to be due in large measure to depression of the center's responsiveness to its own chemical stimulus; we did not believe that chemoreceptor reflexes are an important factor in maintaining eupneic respiration or in bringing about the respiratory response to carbon dioxide under ordinary conditions.

It is on this last point that a difference of opinion exists. As we have pointed out elsewhere (165), since nobody denies that carbon dioxide can act both on the center and on the chemoreceptors, the data required to determine the part played by each of these in the organism's response to carbon dioxide must be adequately quantitative. Unfortunately there is at present a deficiency of suitable

quantitative data; much of that which exists was contributed by this laboratory and it led us to draw the above-mentioned conclusion, which was based on experimental observations and not on preconceived ideas. Several recent papers contain arguments against our view, but the evidence does not seem to us to justify the conclusions drawn. Two of these (67, 68) are reports of studies of electrical disturbances produced in the sinus nerve of the cat by anoxemia, hypercapnia, and various drugs. We have already pointed out (165) that since no evidence has as yet been presented to prove that the electrical activity under investigation actually originated in the chemoreceptors, it is scarcely proper to use the results of such studies as evidence of the sensitivities of these structures. These workers (68) have come to suspect that some of the small action potentials that had been supposed to represent chemoreceptor activity actually may arise in the pressoreceptors of the carotid sinus; it was because we could not get rid of pressoreceptor activity that we abandoned attempts to repeat these observations in dogs (165, p. 117). As a matter of fact, even if one concedes that these potentials were entirely due to chemoreceptor activity, the results indicate that the reflex system of the anesthetized cat must be more sensitive than that of man or dog, so that general application of the conclusions is precluded. Thus the electrical discharge began as soon as the oxygen saturation of arterial blood fell below 96 per cent, from which the authors conclude that a continuous chemoreceptor drive is to be expected from the oxygen unsaturation ordinarily present in many normal persons; the fact that oxygen inhalation by normal man does not depress his breathing shows that this is not true; in a recent study (175) oxygen inhalation was found stimulant rather than depressant to breathing. The authors' further conclusion, from their action potential studies, that the carbon dioxide tension existing during eupnea maintains a continuous chemoreceptor drive, is not borne out by experiments in which respiratory activity and arterial carbon dioxide tension were measured in lightly anesthetized dogs (166). Further work is needed to determine whether these discrepancies are due to species differences, to differences in anesthesia, or to contamination of the action potentials by the activity of something other than the chemoreceptors.

Another paper in which the conclusion is reached that chemoreceptor reflexes play a significant part in the maintenance of

eupneic breathing is that of Gesell, Lapides & Levin (81), who estimated chemoreceptor activity by the extent to which the breathing of vagotomized, anesthetized dogs was depressed when both sinus nerves were blocked by cooling. Respiration was depressed by this procedure whether the animal was breathing air containing 40 per cent oxygen, room air, or air deficient in oxygen; this was taken to mean that at the oxygen unsaturation associated with eupnea at sea level there is considerable chemoreceptor activity—a conclusion that apparently cannot be applied to normal man because his breathing is not depressed by inhalation of oxygen, a fact which strongly suggests deep anesthesia by chloralose as an important factor in the results obtained; no data bearing on the oxygen saturation of the blood are given. The only condition under which blocking the nerves did not cause respiratory depression apparently was inhalation of fairly high (6 per cent or more) concentrations of carbon dioxide; this is taken to mean that high carbon dioxide tensions block the transmission of impulses from the chemoreceptors to the center. We do not see why this result cannot with equal propriety be explained on the very different basis that since carbon dioxide is known to be a relatively weak stimulant to the chemoreceptors (28, 53) but a very strong one to the center, a progressive rise in the carbon dioxide tension of the arterial blood would naturally increase the central component more than the reflex and a time might well come when the relatively weak reflex factor could be eliminated without its absence being noted in the presence of an overpoweringly strong central stimulation. One of the deductions drawn by Gesell from these observations is that anoxemia is rendered more effective as a stimulant to respiration by the reduction in arterial carbon dioxide tension associated with it; the more general belief is that the loss of carbon dioxide weakens the respiratory (100) and vasomotor (74) stimulant effects of anoxia because it reduces the chemical stimulus to the center. Marri & Hauss (134) conclude that the reflexes from the carotid pressoreceptors are made less effective by anoxemia, and several workers (62, 74) have recently presented evidence to indicate that the effects of anoxemia are intensified by increasing the carbon dioxide tension of the blood. Gesell also concludes that the chemoreceptors are continuously activated to an important degree by the arterial carbon dioxide tension of eupneic breathing but this conclusion is at variance with that derived from experiments in

which actual data were obtained with respect to the chemical composition of the blood in animals whose respiratory centers were proved not to be profoundly narcotized (166). Since no corresponding data are presented by Gesell we are unable to decide whether the two sets of observations are comparable or not. The evidence in his paper is limited to three sets of kymographic records; no mention is made of the number of experiments performed or of the scattering of the individual observations. Such statements as these: "There was either a diminution (in breathing) or no effect at all" and "Our own orientation experiments already cited indicate the same for at least a portion of the animals," leave some doubts as to the consistency of the phenomena illustrated. For these reasons we do not feel inclined as yet to modify our earlier position.

The status of the hydrogen ion as a stimulus to center and chemoreceptor is at present quite uncertain. The sensitivity of the center apparently is much lower (146) and that of the chemoreceptors considerably higher (165) than had been supposed. At the moment it seems quite possible that the respiratory effects of changes in pH, in so far as they are not referable to corresponding changes in carbon dioxide tension, are entirely due to chemoreceptor reflexes and not to a direct effect of hydrogen ions on the center, but this remains to be proved.

Other recent work on the subject of carotid and aortic body reflexes includes the morphological studies of Hollinshead (103), Goormaghtigh & Pannier (84), and Addison & Comroe (2). Euler and co-workers (68) and Mosco (140) have studied the actions of lobeline on these structures and the latter has proposed lobeline as a clinical test of circulation time. Stella (180) found that stimulation of the carotid chemoreceptors caused an increase in "apneustic" breathing. Verdonk (187) studied chemoreceptor reflexes in the monkey and reported results similar to those already familiar from experiments on other animals. Shen & Hauss (174) found that dinitrophenol, dinitrocresol, and paranitrophenol, which are characteristic stimulants of cell metabolism, can stimulate the carotid chemoreceptors, their effectiveness being in the order listed, which is also the order of their potency as metabolic stimulants. They attribute the result to increased metabolic activity of the chemoreceptors. A nonspecific irritant action was excluded by the negative outcome of similar tests with phenol and picric acid, which are not metabolic stimulants. Partridge (150) concluded,

from studies of action potentials and of the effects of electrical stimulation of the vagus nerve in cats, rabbits, and dogs, that the fibers responsible for the acceleration of breathing that occurs on stimulating this nerve are those from the cardiac region, not from the lungs; these fibers apparently are quite small and probably include those from the aortic body, which Comroe (52) found to join the vagus in the region of the origin of the recurrent laryngeal. Partridge believes that the acceleration of breathing, observed by Hammouda & Wilson (95) when the vagus trunk was stimulated on the cardiac side of a subtotal block produced by cold, is due to excitation of fibers from the cardiac region (presumably the aortic body). De Bettencourt (29) attempts to account for carotid body reflexes through liberation of a chemical agent, but the evidence is not impressive. Grandpierre (88) presents a review of the literature and a report of a few experiments confirming Heymans' view (now generally accepted) that inhalation of nitrogen no longer causes hyperpnea after denervation of the carotids and aorta.

The reflexes from the pressoreceptors of the carotid sinuses have also received some attention. Benard & Merklen (22) report that the fall in blood pressure produced by pressure on the skin over the carotid sinus is not entirely due to carotid sinus reflexes because some of it is retained after denervation of the sinus. Novak (149) describes the technique for denervating the carotids and aorta in the dog and reports results indicating that failure to get sustained hypertension after this procedure is probably due to incomplete denervation. Hahn (91) states that such denervation regularly caused a considerable (10 to 35 per cent) increase in the oxygen consumption of anesthetized dogs—a finding which he ascribes to removal of a tonic inhibitory control exerted by the reflexes on metabolism; he also found a sustained increase in respiration amounting to about 10 per cent, which would indicate that removal of the pressoreceptor reflexes was dominant over loss of the chemoreflexes in these animals. Marri & Hauss (134) conclude that epinephrine apnea is due to pressoreceptor reflexes unless blood pressure is low or morphine is given. They also find that the respiratory reflexes from the pressoreceptors are depressed by anoxia, by hypercapnia, by hemorrhage, by eserine, by barbital, and by evipal, while morphine increases their effectiveness. Analogous experiments in this laboratory (163) have given essentially similar results as far as the barbiturates are concerned but en-

hancement by morphine was not consistently observed; one interesting observation was the uniform absence of apnea on electrical stimulation of the fibers from the pressoreceptors of the carotid sinuses in decerebrated dogs, although the rest of the reflex (hypotension, bradycardia) was present and apnea regularly occurred on similar stimulation in anesthetized dogs. The significance of this observation is not evident at the moment. Sprenger (176) concludes that the hyperpnea produced by removal of a clamp placed ten minutes previously upon the abdominal aorta is due to reflexes from the pressoreceptors since it is absent when they have been inactivated.

PHYSIOLOGICAL PROBLEMS RELATED TO AVIATION

Armstrong's book (6) has served as a most timely and badly needed source of information on these points. The problems of chief importance to respiration can conveniently be subdivided into: (a) the effects of anoxia; (b) poisonous effects from oxygen; (c) the effects of a rapid decrease in barometric pressure.

(a) *The effects of anoxia.*—The symptoms of acute and chronic anoxia as encountered in aviation are described by Armstrong (5, 6). He attributes them entirely to decreased partial pressure of oxygen in the inspired air; the duration of exposure seems as important as the degree of anoxia (5). Strughold (183) also describes these symptoms and explains them similarly. The importance of decreased tension of carbon dioxide in the blood as a factor in these effects is denied by French authors (26, 33); inhalation of carbon dioxide however is said to abolish Cheyne-Stokes breathing and to improve cerebral functions in aviators (26). Rühl & Kühn (161), claim that the increased oxygen consumption ordinarily seen during exposure to low barometric pressures can be diminished by the addition of 2 to 4 per cent of carbon dioxide to the inspired air. Dill, Edwards & Robinson (61) reported results which offer some support for Mosso's belief that loss of carbon dioxide consequent on the lower barometric pressure is an important feature in altitude sickness. They found that the arterial carbon dioxide tension in four normal subjects was lower when they were breathing, at a total pressure of 435 mm. Hg, a mixture containing enough oxygen to make its partial pressure about 90 mm. Hg, than when they were breathing mixtures containing oxygen at the same partial pressure but at sea level. The difference was not attributable to a

difference in pulmonary ventilation or oxygen consumption; carbon dioxide evidently leaves the blood more readily at lower barometric pressures, as claimed by Mosso. The authors suggest that this may be because better mixing of the alveolar and dead space air occurs when their density is reduced. Luft (124) describes some of the experiences of the German expeditions to the Himalayas; he points out that men have climbed on Mt. Everest higher than 8,500 meters (27,600 feet) without oxygen, indicating the extent to which acclimatization can attain.

The altitude at which anoxic symptoms first appear, though subject to wide individual variations, generally lies in the vicinity of 6,000 to 10,000 feet (6). In some individuals respiratory stimulation begins even at 4000 feet (6) which corresponds with inhalation of 18 per cent oxygen at sea level; this confirms the results obtained some years ago by Ellis (64). The increase is more in depth than in rate (6). Acceleration of the pulse may also begin at 4,000 feet (6); Benson (23) found it first occurring at 8,000 feet in his subjects, and sometimes it does not begin until an altitude of 12,000 feet is reached (6). Subjective symptoms (sleepiness, headache, fatigue, euphoria) usually begin at about 10,000 to 12,000 feet (6). According to Strughold (183) the cerebral functions are the first to suffer obvious impairment and the critical altitude for this is about 14,600 feet (4,500 meters). Armstrong (6) and Strughold (183) re-emphasize the insidious nature of these symptoms because pain is characteristically absent and the ability of the brain to perceive and act upon the growing impairment of body functions is reduced from the start. Jokl (106) reports that reflexes are first diminished (at 13,000 to 15,000 feet), then increased (15,000 to 29,000 feet), and finally (above 30,000 feet) convulsions may occur. The precise cause of these convulsions is unknown at present; perhaps, like the convulsions occasionally seen in perfusion experiments on animals (165, p. 147) they are due to chemoreceptor reflexes. According to Strughold (183) the danger zone lies at the level at which the arterial blood is only 80 per cent saturated with oxygen and this occurs at 13,000 to 16,000 feet; above 20,000 to 22,500 feet there is critical danger to life and unless oxygen is given very soon convulsions will occur and coma and death will follow. The time reserve during which effective action is still possible if the oxygen supply is suddenly interrupted at 22,500 feet, according to Strughold, is less than one minute. Armstrong (6) gives a wider margin of safety:

coma comes on in fifty seconds at 38,000 feet, in ninety seconds at 30,000 feet.

The extremely serious effects of breathing carbon monoxide at high altitudes are emphasized by Strughold (183) and Armstrong (6). Heim (97) notes that inhalation even of 0.01 per cent of carbon monoxide at 10,000 feet reduces the oxygen-carrying capacity of hemoglobin by 10.5 per cent and thus produces considerable aggravation of the anoxia already present. In this connection the experiments of Jongbloed (107) are of interest: using an electrical method for which great precision is claimed, he found that the alveolar air of smokers contained 0.001 per cent of carbon monoxide in the morning before smoking, and that this concentration rose steadily with each cigarette or (particularly) cigar. Apparently carbon monoxide is eliminated relatively slowly and the alveolar tension of the gas rises steadily during the day in those who smoke and in those who are exposed to carbon monoxide from this and other sources.

Gellhorn and his collaborators (73, 74, 75, 113, 196) have continued their studies of various physiological effects of anoxia, but since these do not deal with respiration they need only be mentioned here. Quastel (155), in commenting on the current successful employment of apparently very different procedures (narcosis, insulin shock, metrazole convulsions, and nitrogen inhalations) in the treatment of schizophrenia in man, concludes that the common factor is probably inactivation (temporary or permanent) of the responsible nerve cells by interference with the oxidative processes occurring within them. According to this view the similarly beneficial effects of electrical shocks would have to be attributed to cerebral anoxia due either to respiratory arrest or fall in blood pressure, or perhaps both. Earlier work indicating that metrazole causes cerebral anemia (116) has not been substantiated (121), and experiments (unpublished) in this laboratory have shown that its characteristic effect on cerebral blood flow is a pure increase. McQuarrie and collaborators (130) report results which suggest that an increase in plasma potassium content plays an important part in the convulsant effects of insulin and anoxemia; the report is preliminary, but it is interesting to note that potassium is also a stimulant to the chemoreceptors (165).

The effects of anoxia in aviators are of course reduced or abolished by the inhalation of oxygen. The mask devised by Boothby,

Mayo & Lovelace (38) has been found very effective though the discomfort of all such appliances makes it difficult to get fliers to use them until the discomfort of anoxia becomes burdensome. Behnke (21) states that 8,000 feet is the highest permissible altitude for prolonged exposures without oxygen; brief exposures to 12,000 or 15,000 feet may, however, be tolerated without serious harm. Armstrong (6) cites the following regulations for military aircraft in this country: oxygen inhalation is required at all flights above 15,000 feet, at 12,000 to 15,000 feet when the exposure lasts two hours or more, and at 10,000 to 12,000 feet if the exposure lasts six hours or more. The difficulties encountered, particularly in civilian flying, are probably to be solved only by the use of pressure cabin aircraft.

Beginning or resumption of the inhalation of oxygen at high altitudes sometimes produces unpleasant or dangerous effects, which apparently are due to sudden removal of a strong reflex stimulus set up by anoxemia. Schwarz & Malikiosis (171), when they tested the effects of oxygen inhalation on their subjects after prolonged exposure to low barometric pressures, found that symptoms such as partial or complete unconsciousness or clonic movements of the hand were sometimes seen as cyanosis disappeared; on further investigation they found that in most of these cases a distinct fall in blood pressure and slowing in pulse rate occurred as soon as oxygen was inhaled. Jokl (106) reports similar experiences. The explanation of this rather remarkable phenomenon is not at present available. The reviewers wonder if the situation may not be similar in some respects to that existing in those patients who die when a severe tracheal obstruction is suddenly relieved by tracheotomy (143). In such cases it is probable that anoxia of sufficient severity and duration to produce extensive damage to brain cells was already present but the system was kept active by the continuous play of strong excitatory impulses aroused in the chemoreceptors by the anoxemia. Sudden relief of the latter would then remove the factor that had kept the system going, before recovery from the depressant effects of the anoxia could occur. It is also possible that the phenomenon as it occurs in aircraft is bound up with the vigor of the hyperpnea that preceded the oxygen inhalation; spasm of cerebral vessels due to reduction in arterial carbon dioxide tension, minimized by the cerebral vasodilator effects of anoxemia but brought out in all its intensity when

the latter is suddenly removed, may also be considered. The fall in blood pressure reported (171) in such cases suggests that the vasomotor center was considerably depressed but that this depression was being counteracted by reflexes aroused by anoxia (52). This onset of dangerous effects when oxygen is breathed after severe anoxemia has lasted for some time may not be as rare as the paucity of information about it now indicates it to be; Schwarz & Malikiosis cite the case of a flier who discontinued oxygen inhalation for a few minutes at about 20,000 feet to make an adjustment, and who then collapsed unconscious when he resumed the oxygen inhalation. Something like this happening to a pilot and leading to a crash would be blamed on failure of the plane unless it was actually seen not to be (H. C. Bazett, personal communication). Obviously this is another argument for using oxygen before anoxemia appears.

The ceiling for safe flight with inhalation of oxygen but without increasing its pressure above that of the surrounding atmosphere, according to Armstrong (6), is 30,000 feet under any but the most unusual circumstances, and nobody should ever be allowed to fly above 40,000 feet. The partial pressure of oxygen in the alveolar air (assuming the inspired gas to be 100 per cent oxygen) would be equal to that normally existing at sea level (100 mm. Hg) at about 33,500 feet, but Armstrong (and Heim) found that the oxygen saturation of arterial blood began to fall below normal at 27,500 feet for unknown reasons. Perhaps the inhaling devices were not 100 per cent efficient. At about 50,000 feet the existing atmospheric pressure (86 mm. Hg) would equal the sum of the tensions of water vapor and carbon dioxide in the alveolar air ($47+39$ mm. Hg at sea level) and oxygen would not penetrate to the alveoli even though the inspired gas consisted entirely of oxygen. This would be absolute ceiling for flight with oxygen were it not for the fact that the alveolar carbon dioxide tension certainly, and the water vapor tension very probably, would be reduced at the higher altitude. At 40,000 feet the oxygen saturation of arterial blood of a subject breathing 100 per cent oxygen would be about 88 per cent, or about the same as that found while breathing air at 12,000 feet (6); the great danger would be that in the event of sudden failure of the oxygen supply at 40,000 feet, consciousness would probably be lost in a few seconds. A portable individual supply of oxygen, suitable for use under such circumstances or if it is necessary to

"bail out" of a plane at a high altitude, has recently been described by Boothby *et al.* (39).

(b) *Oxygen poisoning*.—Ever since the work of Paul Bert it has been known that pure oxygen under increased pressure can produce fatal poisoning, characterized in experimental animals by pulmonary irritation and edema and violent prolonged convulsions. Bean (17) has recently studied the possible cause of these events and has concluded that the convulsions are due to accumulation of acid in the brain cells because oxyhemoglobin is not reduced and alkali is therefore not made available for the transport of carbon dioxide away from them. If that were true the carbon dioxide tension of the venous blood effluent from the brain should be greatly increased under such circumstances, but there is no evidence to that effect. Bean (16) has recently found that acid does not accumulate in intestinal muscle exposed to oxygen under five atmospheres pressure, but states that the situation in the body may be different. It seems most probable (as Bean states) that oxygen poisoning is related to an interference with or perversion of intracellular oxidative systems, but the nature of the change, and whether it is cause or effect, remain to be determined.

The possibility of poisonous effects from inhalation of pure oxygen is not a problem of aviation, for oxygen is not used here until the atmospheric pressure is so low that the partial pressure of the gas in the alveoli is little if at all above that while breathing air at sea level. The question has great importance for divers and caisson workers, however, and the recent availability of 100 per cent oxygen inhalations for various clinical conditions has further increased the interest in this problem. Boothby *et al.* (38) report no harmful effects from inhalation of 100 per cent oxygen for periods as long as two days. This is contrary to the experience of several recent workers (19, 20, 30, 98). Behnke in particular (20) emphasizes that normal healthy men cannot breathe pure (99 per cent) oxygen for more than six to seven hours without symptoms such as nausea, substernal pain, and flushing of the face. One subject (19) developed repeated vomiting followed by a frank bronchopneumonia. It is to be emphasized that these results can be obtained only by inhalation of pure (or nearly pure) oxygen at sea level; at or below concentrations of 60 per cent the inhalation of oxygen apparently is quite harmless. It is possible that patients

with anoxemia are more resistant to the poisonous effects of pure oxygen (20).

(c) *The effects of decompression.*—Armstrong (6) suggests the term "aeroembolism" for the condition produced by the liberation of gases from solution in the body fluids consequent on rapid reduction in barometric pressure in a rapidly ascending airplane. According to Armstrong (6), there is a critical level at about 30,000 feet; below this level aeroembolism is not likely to occur no matter how rapid the ascent but above it the ascent must be very slow if danger is to be avoided. Experimental animals are more resistant, for gas emboli do not occur unless the barometric pressure is quickly lowered to 60 mm. (about 58,000 feet) or less (170). Behnke (20) points out that an aviator ascending from sea level to 33,800 feet (at which the barometric pressure is one fourth that at sea level) is in exactly the same state as a diver ascending in salt water from 100 feet (at which the pressure is four atmospheres) to the surface. The symptoms of such decompression vary from mild disturbances, such as pruritis, especially over the chest and abdomen and in the lobes of the ears, transitory macular eruption, and pains in the joints, particularly in the knees, through severe manifestations, including substernal and precordial pain, probably due to pulmonary emboli, and symptoms referable to emboli in the central and peripheral nervous system, to fatal coma and shock, from embolization of the cerebral circulation and the presence of gas in the cardiac chambers. These effects are relieved by recompression but not by oxygen.

As to the gas liberated from the blood on decompression, Behnke (20) states that considerable of it is carbon dioxide, while Schubert & Grüner (170) believe that it consists mainly of carbon dioxide and oxygen, nitrogen being relatively unimportant. The latter find that the animals used, rats, tolerate decompression much better if they were made to breathe oxygen for some time before the experiment but this is attributed to greater ability to withstand anoxia because of greater stores of oxygen. They found rats more susceptible to sudden decompression than guinea pigs, cats, and dogs. Engelhardt (65) investigated the rate at which nitrogen is eliminated from the body during oxygen inhalation and concluded that it occurs at the rate of about 40 to 80 cc. per minute or 3.3 to 6.7 cc. per breath. He calculates the total amount

of physically dissolved nitrogen in the body at sea level to be about 750 cc.

This question of sudden decompression has come to be a very important one in connection with the use of pressure-cabin aircraft, for explosion of such a cabin at an altitude of 40,000 feet would mean decompression of the order of 600 mm. Hg in a fraction of a second (71). In one set of experiments intended to simulate these conditions, thirty-one out of thirty-three animals showed no ill effects (71); the two that did became ataxic and showed paralysis of the hindquarters for a short while but they soon recovered. It is perhaps suggestive that the only animals affected were pigs, which probably had a high fat content; three other pigs, six monkeys, six dogs, four chickens, ten rabbits, one cat, and one guinea pig were free of symptoms. If it is true that susceptibility to aeroembolism varies widely in different species of animals, deductions with respect to man must be drawn with caution. Bergeret & Giordan (27) performed a similar set of experiments on anesthetized dogs and found no changes in blood pressure on explosive decompression; respiration was increased in rate but decreased in depth, presumably because of sudden expansion of intestinal gases. The only serious results were hemorrhages into the middle ear, which occurred in nineteen out of twenty-two animals, without, however, rupture of the drum; hemorrhage also occurred in some animals around the petrosal sinuses. Because of the dangers incident to sudden decompression, Armstrong (6) believes that planes with sealed cabins should not operate at a height greater than 30,000 feet.

According to Berg *et al.* (25) the hydrogen ion concentration of the blood of normal men and dogs is subject to wide spontaneous fluctuations (from pH 7.28 to pH 7.68) from week to week and these fluctuations show close correlation with changes in barometric pressure; the blood samples were collected from cutaneous veins and the observed changes must therefore have been dominated by changes in the peripheral circulation. Flying is peculiarly dangerous for patients with pneumothorax (72).

MISCELLANEOUS TOPICS

Studies of alveolar air have recently been made by Mackay (127, 128) and Cotton (55). The former was unable to confirm the classical (and almost universally accepted) result of Haldane &

Priestley, for he found a plateau in the carbon dioxide content of air expelled from the lungs in only one out of thirty-three determinations in six subjects; he concludes that samples taken from the air expelled from the mouth do not necessarily conform to changes occurring in the ventilating surfaces of the lungs. Cotton (55) found a paradoxical rise in the oxygen content of expired air as the expiratory act proceeds; he believes that the highest value is the true one for alveolar air; of six subjects studied, five showed this type of effect.

Stimulation of the central end of one vagodepressor nerve of the dog was carried out by Boyd & Maaske (41) who concluded that the fibers which lead to acceleration of breathing are identical with those which cause inhibition, the acceleration being produced in some way by reduced inhibitory activity. Gesell (78), on the other hand, maintains that the vagus is essentially an excitatory nerve to respiration. Hamilton & Gesell (94) have made a preliminary report of experiments in which vagus stimulation was inhibitory to breathing; impulses from cutaneous sensory nerves were able to overcome this and produce rhythmic breathing. Graham (87) has studied the nature of pulmonary ventilation in the fowl and the influence of the vagus nerve upon it; the pulmonary gas exchange apparently is accomplished by inspiration of air into the abdominal air sac whence it is expired into the thoracic air sacs; most of the gas exchange occurs during the expiratory phase; the vagi serve more to promote expiration than to inhibit inspiration as in mammals. Wyss (195) also stimulated the vagus in the rabbit and found that the nature of the effect on respiration depended on the frequency of the stimulus: at low frequencies (about 50 per sec.) purely inspiratory effects were obtained, at high (320 per sec.) purely expiratory, with a transition from one to the other at about 70 to 120 per sec. He believes that the inhibitory effect is the only one of physiological importance, the excitatory response being due to opening of a new central path the nature of which is to be the object of further study. Bilateral vagotomy is followed by atelectasis and consolidation; perhaps the vagi control the permeability of the alveolar membranes (76).

Studies of other reflexes affecting respiration suggest that afferent impulses involved therein may be conducted in the phrenic (45, 101) and intercostal nerves (108). Dufour (63) claims that reflexes from the pleura are vitally important to the maintenance of

respiration, but the course of events described by him strongly suggests that severe pneumothorax was responsible for the results.

The influence of anesthetics on various respiratory phenomena was studied by a number of workers. Benzinger, Opitz & Schoedel (24) confirm the observation (53, 135, 165) that narcotics depress the response of the center to carbon dioxide more than that of the chemoreceptors to anoxemia. In decerebrate dogs chloralose exaggerates the effectiveness of chemoreceptor reflexes and reduces that of carbon dioxide on the center; ether depresses the reflex response more than the central, while barbital is intermediate in action (163). Barbiturates decrease the effectiveness of carotid sinus pressoreceptor reflexes on respiration in dogs while morphine exaggerates it. The recent vogue of intravenous anesthesia by pentobarbital entails special hazards because respiration is apt to be severely depressed by full anesthetic doses; occasional inflation of the lungs with oxygen containing a little carbon dioxide greatly increases the safety of this procedure and is preferable to the use of analeptic drugs (132). Metrazole has strong but brief respiratory stimulant effects in dogs deeply poisoned with ethyl alcohol; the rate of disappearance of the latter is not influenced (126). SeEVERS and his colleagues have studied the effects of hyperventilation during anesthesia; they made the interesting observation that the fall in blood pressure associated with overventilation occurs only in the presence of general anesthesia (172), so that the acapnia theory of shock is tenable only for the anesthetized individual. Even very severe and prolonged overventilation, lasting as long as fifteen hours and reducing the arterial carbon dioxide to 9 vol. per cent, is seldom fatal to anesthetized dogs; spontaneous respiration is usually resumed after the overventilation ceases. Hemoglobinemia usually occurs after overventilation (182). Hyperventilation of human subjects under general anesthesia also caused a fall in blood pressure (173); since this is not the case in unanesthetized individuals, it is evident that anesthesia deranges some mechanism by which the vasomotor center is protected against the effects of reduction in arterial carbon dioxide tension. The nature of this mechanism has not been ascertained; readjustment of the medullary circulation has been suggested (167).

Studies pertaining to the respiratory functions of the blood were made by ETTORI & GRANGAUD (66) who report that the volume of human erythrocytes was not significantly altered by changes in

carbon dioxide tension over the range of 15 to 60 mm. Hg if the hemoglobin was entirely reduced; at 400 mm. Hg a slight increase in cell volume was noted. When hemoglobin was entirely oxygenated an increase of carbon dioxide tension over the range of 0 to 100 mm. Hg caused a slight increase in cell volume. When the carbon dioxide tension was kept constant at 40 mm. Hg, changing the oxygen tension from 0 to 100 mm. Hg caused a reduction in cell volume, i.e., an effect opposite to that of carbon dioxide. Irving, Black & Safford (104) report that the erythrocytes of the trout swell as much as 20 per cent when the carbon dioxide tension is changed from 1 to 10 mm. Hg; the combination of oxygen with this hemoglobin is very sensitive to changes in carbon dioxide tension and temperature. Binet & Strumza (32) find that the resistance of anesthetized dogs to severe anoxemia is significantly enhanced by mobilization of erythrocytes from the spleen; the increase in erythrocyte count and hemoglobin content may be considerable (18 and 16.5 per cent respectively) and after this has occurred the animal's resistance to anoxemia is increased about 30 per cent. Luckner (123) has studied the kinetics of the combinations of oxygen and carbon dioxide with hemoglobin; he concludes that the gas exchange in the capillaries of the lungs or tissues requires about one second and calculates that only 0.8 to 1 sec. is ordinarily available for the purpose; any acceleration of the circulation would therefore tend to make the gas exchange incomplete. Nims *et al.* (148) report that patients subject to epileptic seizures are less able than normal persons to protect their brain cells from reduction in carbon dioxide tension during hyperventilation.

Acclimatization to high altitudes is described by Luft (124) as a result of experience in the Himalayas. Acclimatization to increased carbon dioxide tension was studied by Miller (136) who exposed normal dogs to 1.5 to 5 per cent carbon dioxide for periods of as long as four weeks and found in most cases a fall in alkaline reserve, a drop in blood chloride and an increase in erythrocyte count and hemoglobin; the blood changes are similar to those produced by anoxemia, probably because an increase in tissue acidity and a decrease in tissue oxidations occur in both instances.

Carbon monoxide may produce chronic poisoning and be responsible for a variety of symptoms that are not ordinarily blamed upon this agent (18). Methylene blue assists in breaking down the

compound of carbon monoxide with hemoglobin (31). It does not however assist in combating monoxide coma in rabbits; breathing is stimulated by carbon monoxide in these animals (59) which is not usually the case in man. A case of severe and prolonged poisoning by carbon monoxide in man, with eventual recovery, is described (162). Stannard (177) reports results indicating that carbon monoxide not only inhibits the activity of cytochrome oxidase in frog muscle, but also can be oxidized to carbon dioxide by this tissue by a system that has not yet been identified. The literature on carbon monoxide has been reviewed by Killick (109).

The effects of respiratory movements on the circulation were studied by Cahoon, Johnson & Michael (44), who concluded that cardiac output is decreased during inspiration, due apparently to decreased filling of the heart as a result of expansion of the auricles. A violent expiratory effort with the lungs full of air causes marked diminution in the pulse transmitted to the air in the respiratory passages (141). The respiratory and circulatory adjustments that occur in a diving mammal on submerging are described by Krogh (114); further studies of these mechanisms in the seal are reported by Scholander (169). Trimby & Nicholson (184) report that the respiratory waves in blood pressure are due to changes in capacity of the pulmonary vascular bed, an increase with inspiration being responsible for a drop in systemic pressure.

Studies of the comparative physiology of respiration include Krogh's book (114), investigations of thoracic movements in the lizard (35), of respiratory movements of a crab (86), of the air stream in the fowl (87), and of the possibility of obtaining enough oxygen from water to support life when submerged (154).

Essays pertaining to the regulation of respiration were contributed by Anthony (4) and Gesell (78). Koppanyi & Linegar (111, 112) believe that the response of the chemoreceptors to acetylcholine has physiological importance; this view is not shared by the reviewers, mainly because the amounts of this agent required to produce definite chemoreceptor responses, even under circumstances as favorable as possible for demonstrating such action, are greatly in excess of any that could conceivably be present under physiological conditions. In our opinion not enough is known about the mode of action of any of the drugs used by these authors to justify the hope that useful additions to our knowledge can be made by using them in various combinations.

The influence of training on the efficiency of the respiratory exchange was studied by Schneider & Crampton (168); they found that the volume of air that had to be breathed for each 100 cc. of oxygen absorbed was definitely higher in the untrained subjects.

Fetal respiratory functions have been studied by a number of workers, Windle and his collaborators and Barcroft's group having been particularly active. The former believe that the fetus does not normally make respiratory movements *in utero* but does so only when its oxygen supply is reduced to an abnormal level (190); they also hold that the pulmonary circulation passes about as much blood in the unexpanded fetal lung as it does afterward (1, 191); Windle's book (192) contains an excellent summary of existing ideas on this subject. Barcroft (11) sketches the development of respiratory control in the fetus: the reflex regulation becomes effective before the chemical but as term approaches inhibition of respiratory movements by the higher centers becomes more and more marked; the brain of the fetus is subjected to an oxygen tension about the same as that encountered by dwellers at very high altitudes, but in the last week before birth a sharp drop occurs (12); the blood in the umbilical vein is about 70 per cent saturated with oxygen until the last week of pregnancy when it falls rapidly and irregularly (13). The respiration of newborn rabbits can be stimulated by lobeline and cyanide but convulsions occur when the dose of lobeline is double the minimum effective one; these convulsions are apt to be fatal, and this is also true with caffeine and coramine (117). Neonatal asphyxia in humans is contributed to by trauma, asphyxia and narcosis (50). In resuscitation of the newborn, intubation of the trachea and insufflation with oxygen plus carbon dioxide is very effective (69),

The respiratory functions of the lungs were investigated by Asmussen and co-workers, who found no change in the mid-capacity during exercise (7); the vital capacity tends to be greatest when the legs contain most blood, least when the major vessels are fullest, the difference amounting to nearly 300 cc. and being due to variations in the amount of blood in the pulmonary circulation (9). In support of this, venesection has been found to produce a distinct increase in vital capacity (83). The rhythmic changes in bronchial caliber associated with respiration are probably purely passive and not reflex in origin (144). There is no effective lymph drainage from the alveoli (120). The fatal effects of the blast of

high explosives are due to traumatic rupture of pulmonary blood vessels and hemorrhage (197). The capillary area in the lungs is probably subject to change (70).

The effects of oxygen, other than the relief of anoxic symptoms in aviators and athletes (see above), have been tested in patients with myocardial infarction and shock, with encouraging results (37, 194). The retinal vessels dilate during anoxia (corresponding to an altitude of 18,000 to 21,000 feet) and are constricted during inhalation of high concentrations of oxygen (56); the cerebral vessels are supposed to behave similarly. Oxygen absorbed through the skin may account for as much as 10 per cent of the total metabolic use of the gas (46). Respiration of normal young adults is quite consistently stimulated by inhalation of oxygen (175); the cause of this hyperpnea is still unknown. The oxygen tensions existing in the tissues, measured during bed rest, were found by Del Baere (58) to be lower (2.6 to 2.9 per cent) in cases of cardiac failure than in normal subjects (5.5 to 9.6 per cent). The oxygen tension in a peritoneal gas pocket was lowered during hibernation in the ground squirrel (181). Asmussen *et al.* (8) measured the oxygen consumption by the leg muscles of man by noting the reduction in total oxygen uptake produced by obstructing the circulation to the legs; they concluded that an increase to 112 times the resting level occurs during strenuous work by the legs, while with the maximum possible work the increase would amount to 180 times that at rest; the blood flow through the muscles would have to increase at least fiftyfold during the work.

Of various drugs tried on patients with Cheyne-Stokes breathing, theophylline and its various combinations were most effective in restoring breathing to normal; since carbon dioxide does not act similarly, the effect is supposed to be on the cerebral circulation rather than a direct stimulation of the center (142).

The effects of the electric current on the lungs were studied by Greenberg (89), who found that the volume of the thorax was diminished, this fact indicating the expiratory muscles to be stronger than the inspiratory.

The function of respiration in the control of body temperature is discussed by Krogh (114). Randall & Hiestand (156), from studies on panting in the chicken, conclude that the panting center is inactive at normal temperatures and can be paralyzed by narcotics without paralyzing the respiratory center. The interrela-

tions among the respiratory, swallowing, and sucking centers were studied by Peiper (151), who believes that the rhythm of the respiratory center may be determined by that of the sucking center and this in turn by the swallowing center when the latter is active.

Among new methods, the following may be listed: adaptation of the dropping mercury electrode to the estimation of the oxygen and hemoglobin content of small volumes of blood (15); a spectroscopic method for determining the entire dissociation curve on 1 to 2 cc. samples of blood (92); a photometric hematocrit (66); an adaptation of the interferometer for the estimation of carbon monoxide in alveolar air (107); an automatically activated pump for the collection of samples of alveolar air in animal experiments (119); a method for determining the gas tensions in mixed venous blood in man (127); a method for estimating the gas exchange of insects (118); a portable inhaler for administering oxygen to patients under anesthesia (132); a light self-contained supply of oxygen for aviators forced to take to the parachute at high altitudes (39); a catharometric method for determining the vital capacity by means of inhalation of hydrogen (129); a method for fractional sampling of the alveolar air (55); a voltametric method for estimating oxygen (110); and methods for determining the residual air by means of inhalation of nitrogen (57, 158).

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PHYSICAL PROPERTIES OF PROTOPLASM

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Protoplasm is commonly the name of substance that is said to be living. Often it more specifically refers to the semitransparent, semifluid material within cellular units, as observed by Dujardin in 1835 and Schultze (142), and named one hundred years ago by Purkinje in 1840 and Mohl in 1846. When exact definition is sought it seems almost impossible to exclude the surfaces of cells, their "inclusions," noncellular material, and products of past life. Indeed, protoplasm is more than substance; it is, like an engine, the site of activities. Protoplasms are very diverse; they are not always semitransparent, or semifluid. A whale or a man is protoplasm. What the term does is to emphasize units that are too small for an anatomist to manipulate or a chemist to analyze.

To some scientists, the physical properties of protoplasm illustrate neat applications of the techniques of physical science to sub-cellular units, while to others the individual methods of observation result in information that is gradually being correlated into considerable descriptions and concepts of vital activities.

Physiologists often become engrossed in investigating the division of labor among organs and tissues of animals, and ignore the fact that single cells, and even smaller masses, perform similar acts. In recent years certain methods have been perfected to the point where the precision of measurement on a single cell is equal to that on a whole individual. Biologists of various stripes may wish to know what functions are being investigated within the boundaries of cells, and how these are related one with another.

Does protoplasm flow, stick, stretch, dissolve, swell, doubly refract? Can portions of protoplasm be isolated without loss of their ability to survive when kept in suitable surroundings? What structures in it can be dispensed with or modified, consistent with survival? What properties change during growth, movement, cell division, metabolism, and death?

What properties are found in most cells of the diversified tissues of one individual; or among diverse kinds of organisms? Which

physical properties are amenable to study in the cells of mammals?

These are some of the questions that protoplasmologists would like to answer. Provisional indications of several of the answers are at present available, such as will be found at the conclusion of this article. Whether they prove satisfying to many readers is hardly a sound basis for judging this young field of biology.

Since the facts at hand depend upon methods of observing them, the physical properties of protoplasm may be classified operationally. Following a textbook of physics, they are optical, mechanical, electrical, and thermal. Later some inferences from combinations of the observations will be mentioned.

The inquiries covered will be limited chiefly to those made upon actually living material. It is a tenet of protoplasmic study that each unit observed should be tested for subsequent survival. Cells after death, models and predictions, will generally be here omitted. Attention will be paid to processes as well as to static properties.

Excluded will be the effects of physical stimuli and conditions, most studies on plant cells and bacteria, most studies of tissue masses, blood, permeability, staining and fixing. It will also be necessary to omit studies of muscle cells, whose physical properties are the best known of any.

Previous reviews have scarcely defined the field as here. Very general summaries are found in recent books of Seifriz (144), Heilbrunn (67), Barnes (5), Lepeschkin (86), Pfeiffer (124), and Gerard (54). Other reviews are restricted to special portions of the field; e.g., ultrastructure (50), centrifugation (63); or to nonliving systems; e.g., water (156), proteins (74). Additional surveys of certain topics are to be found in Volume 8 of *Cold Spring Harbor Symposium on Quantitative Biology*, and in Volume 19 of *Tabulae Biologicae*.

OPTICAL PROPERTIES

OPTICAL DENSITY

For the most part biologists have been content to know that, in thin layers, protoplasm is nearly transparent. Measurements of total absorption and of transmission for each wave length have been only roughly recorded. The difficulty of ascertaining them accurately lies in the considerable reflection, refraction, and scatter-

ing that are to be evaluated. Construction of optical systems that allow of precision for minute units is far from easy.

Visible light.—This reveals in part those granules and droplets that are studied by cytologists in living material. Absorption in certain spectral regions is credited to specific chemical compounds, such as cytochrome. Segregation apparatus, mast cell granules, and many other bodies are made visible by allowing "vital" stains to get into the cytoplasm.

Double absorption (dichroism), ascertained by using polarized light, is found in some protoplasms. Its presence, as in retinal rods and their visual purple, is linked with macromolecular orientations (132).

Ultraviolet.—Ultraviolet light is absorbed differentially as recorded in photographs. By it (2,600 Å) Caspersson (17) visualized details of the chromosomes in living material (intestinal glands of *Helix*, salivary glands of *Drosophila*), and followed minutely certain changes. From the sudden increase in absorption at metaphase he concluded (18, 19) that nucleoprotein is synthesized during this portion of cell division.

Absorption of ultraviolet and various Roentgen radiations is for the most part studied by their lethal effects (e.g., 69, 158). As an approximation, it may be said that the quanta absorbed to kill equal numbers of cells of one size vary inversely as the wave length.

Electron microscopy.—Electron microscopy is becoming available (3, 56, 80, 102, 143, 159). Its use is limited to dried, therefore dead, materials. Photographs of erythrocytes and bacteria have been made, showing complete resolution at magnifications of 20,000.

REFLECTION

Discoveries by means of the Tyndall beam, in the form of the ultramicroscope, were made a generation ago. Dark-field methods are summarized by Oelze (117). Oriented dark-field illumination may be secured by the Spierer lens, described by Seifriz (144). Longitudinal scattering of light (Plotnikow effect) appears in tissue spaces that are injected with water or air and in cell suspensions. Whether this effect reveals aggregates of chain molecules in regular orientations is uncertain (90).

REFRACTION

Index of refraction.—The index of refraction to ordinary light may be ascertained in single cells of regular shapes such as spheres and cylinders (133).

Refraction renders visible many of the microscopic structures of living protoplasm: nucleus, nucleolus, chromosomes, spindle fibers, aster fibers, Golgi bodies (70), and mitochondria. The last two are visible with regularity in most metazoan cells.

Birefringence.—Birefringence, the double refraction of polarized light, has become a popular tool of observation. Differential refractive indices are found in many protoplasts, indicating the presence of oriented aggregates (macromolecules), of sizes between the visible and the molecular. The methods are presented by W. J. Schmidt (131), Boehm (10), and others. The results are reviewed particularly by Schmidt (134), Frey-Wyssling (50), F. O. Schmitt (138), and Buchthal & Knappeis (13).

In general three classes of birefringence may be distinguished: crystalline, photoelastic (including flow), and form. The second is obtained only in response to external or applied deformation; the third is indicated by change of double refraction with substitution of liquid media of diverse indices; the first is the remainder. All are found in some prepared derivatives of organisms. Stretch and flow may be imposed in living cells, as by centrifugation of dividing oligochaete eggs (123), and the birefringence then appears in spindle fibers and in flowing portions.

Birefringence is found in a dozen types of animal cells, and in a dozen kinds of intracellular structures. The latter includes neurofibrils, myofibrils, chromosomes, aster fibers, Golgi bodies, mitochondria, and cilia. Birefringence is notably absent in just as many other cytological structures. Fairly complete lists of occurrences are furnished by Schmidt (131, 134, 135), and Buchthal (13), with more recent additions by Schmidt (136, 137), Monné (108, 109), Speidel (149), and Pfeiffer (122).

Optically polarized elements are frequently found in elongate cells and organelles. But the polarization is not positive (longitudinal) in all, indicating tangential arrays of macromolecules, probably of protein, in some, such as axon (29) and sheath (155) of diverse species (lobster, earthworm, frog). Whether the oriented elements are uniformly distributed or superficial only is rarely evident. Polarized elements are also found in some spherical struc-

tures, often as lamellae at the surfaces of nuclei, membranes of aquatic eggs, and spermatocytes.

DIFFRACTION

Diffraction patterns are formed by beams of diffracted radiation of short wave lengths. The methods used in biological materials are indicated by Schmitt (138). Practically all protoplasts are killed before or during the radiation.

X-ray diffraction.—Recent studies favor the use of x-rays since they are short enough to detect atomic configurations in molecules. In no case does the pattern ascertained overlap that found by polarized light; structures detected by one may be oriented quite otherwise than those detected by the other.

The methods as applied to proteins and other substances of biological interest are presented by Katz (74). Their application to dried cellular materials, especially nerve and its sheath, is described in several other papers (118, 139, 140). Protein is found to be present in myelin sheath; in the presence of water the oriented lipid layers are moved apart to allow as many as thirty-two molecules of water in the interspace.

Electron diffraction.—The finer structures that might be revealed by this diffraction have not been sought in whole cells (138, p. 281).

INTERFERENCE

Interference figures are found in frog nerves (82). These are said to indicate spiral gratings, having periods that vary with axon diameters. Interference veins appear longitudinally in flowing columns of certain plant protoplasts (121). Interference bands are used (141, 157) to ascertain the total thickness of the surface layers in dried ghost erythrocytes.

PRODUCTION OF RADIATIONS

Light.—The emission of visible light from living cells is reviewed by Harvey (64). While most investigation has centered about the chemical input of the transformation, some facts are known concerning the kinetics and intensities. The rise of intensity upon admitting oxygen to *Achromobacter*, measured photoelectrically, shows a latency that is independent of the oxygen tension (28). Long-wave radiations are also emitted by constituents

of protoplasm that fluoresce either naturally or after staining. The techniques of this study are described by Haitinger (58).

Mitogenetic radiation.—This term refers to the invisible rays alleged by Gurwitsch to be emitted during cell division; they are said to influence cellular activities in several measurable ways. Evidence is still accumulating for and against the reality of such radiation (71, 128). Differences of conclusion appear to depend upon the supposed significance of results with diverse detectors, and judgment of whether phenomena that are not always reproducible at will are still general.

SUMMARY

Optical studies of protoplasm reveal not only structures made visible by absorption and refraction of light, but those that reflect, refract, and diffract short-wave radiations. Only visible and longer waves allow examination of living material without known damage. At present, studies of birefringence are most prominent; the macromolecular patterns of many protoplasms, especially in cell surfaces, are being explored. Elongated structures and a few others doubly refract, revealing an ultrastructure of a sort that might result from growth by intussusception in one dimension. Much of this information is incompatible with the hypothesis that protoplasm is a rather structureless mass of random components. Emission of radiations occurs in restricted kinds of protoplasm and is believed to be invisible as well as visible.

MECHANICAL PROPERTIES

PHYSICAL STATE

Traditionally the liquid characteristics of protoplasm have been associated with the term colloidal. How far the properties of protoplasm may be viewed as those of colloidal mixtures may be judged from, for instance, the book of Lepeschkin (86). From many types of cells a quite liquid material may be expressed, as exemplified in the axoplasm of the giant axons in squid. The thin surroundings of this material constitute a solid-like casing. Most cells have such a semipermanent casing, which may become punctured and repaired or replaced.

Most protoplasms are reversibly liquid and solid. The reversal may be easily observed in tissue culture fibroblasts (91), *Amoeba* (103), and sea urchin eggs (24). The amount of liquid at any one

time is said to be a power function of body size in the protozoan *Actinosphaerium*; after amputation of cytoplasm the original ratio is soon restored by redistribution (116).

Liquefaction may be induced in eggs of invertebrates by micro-manipulation (24), or by imposition of hydrostatic pressures of the order of 300 to 400 atmospheres (99, 119, 120). All degrees of liquid and solid states may prevail. When greater fluidity follows mechanical disturbance, the similarity to thixotropy in gels is striking. The readiness to transform phases is a prevalent characteristic of protoplasms.

PHASES

In the visibly empty phase in cytoplasms, termed "fluid" by Lepeschkin (89), the other phases are suspended or emulsified. The relative volumes of materials that differ in specific gravity from the fluid, separable by centrifugation, have been ascertained in eggs of twenty-six marine species by Costello (40): forty to 82 per cent of total substance, with fluid in its interstices, becomes thus separated. In some, the material lighter than fluid (chiefly fatty substance) is considerable. Two or more "heavy" layers may be distinguished by their specific gravities and their particle sizes. The techniques of determining the specific gravities of whole cells and of cell components have been considerably perfected (84, 94). The specific gravities of white blood cells and of blood platelets were re-determined by Löhner (93).

In colloidal systems it is possible for two liquids to aggregate in separate emulsified droplets and to dissolve reversibly (16). This phenomenon, coascervation, has been sought in numerous kinds of living material, and possibly has been found in some, for instance in the hyaline vesicles sometimes formed on the surface of *Paramecium*.

Some of the granules in sea urchin eggs have regular arrangements near the surface (111). They are of uniform size, and unmoved by centrifugation. They disappear during fertilization and other forms of activation in a wavelike order, even in the absence of oxygen (76). Other granules, pigmented, undergo after isolation swelling and shrinking like osmometers (59). The same granules disappear during the surface precipitation reaction, their dissolution hastening it. Recognition of such distinct phases, therefore, tends to support the idea that segregation of functions may exist in single cells.

By centrifugation, "heavy" halves of sea urchin eggs may be separated and discarded without much embarrassment of the remainders (61). In *Amoeba* the loss of crystals and nutritive spheres (heavy) leads to temporary lack of locomotion; these elements then slowly reform and a normal state is regained (148).

Cell casings are regularly separable, sometimes as pellicles. When fixed frameworks are isolated from lysed erythrocytes enough material is present in them to form a superficial layer 120 Å (0.012 μ) thick (51).

TENSION AT CELL SURFACES

A variety of cells has been investigated with respect to superficial forces. Such forces are related to turgor pressure. They may be influenced by the medium and possibly by the procedure of measurement. By the methods of mechanical compression, centrifugation, and flow, values ranging from 0.04 to 3.0 dynes per cm. have been found (65, 107). Oil drops within eggs of *Daphnia* have interfacial tensions of 0.6 to 1.4 dynes per cm., depending, because of their elasticity, on the centrifugal force used (66). In rabbit macrophages that contain droplets of oil, the tension is 2 dynes per cm. (146). When amphibian erythrocytes were pulled between two calibrated glass needles, the maximal tension found was 1.4 dynes per cm.; it was reduced by lysis of the cells (113). Uncertainty exists in crediting all the tension to the superficial layers. Greater tensions are found where crystalline casings are believed to exist.

DEFORMATIONS

It is well known that cells frequently deform in the presence of local forces. Nonmechanical influences that change erythrocytes from discs to spheres were found to be exerted by hydrophobic monolayers of stearate at 50 μ distance (125). Contact of sperm gives rise to protrusions in immature ovocytes of sea urchins (153). Internal factors may change cell shapes, as in the elongations of eggs in first cleavage (30). Osmotic pressures and state of cortex are among the significant factors.

Centrifugation not only elongates cells but fragments them, even into quarters. In sea urchin eggs, nuclei segregate in the lightest quarters; the spindle is centripetal while the chromatin is centrifugal. If halves are separated before fertilization, both develop

either parthenogenetically or after fertilization. The forces obtainable limit the fragmentations to large cells such as eggs (61) and protozoa (148). Higher temperatures favor the centrifugal separation of halves in sea urchin eggs (39).

Ultracentrifugation displaces most of the visible contents of cells. In spinal ganglion neurons of rats it distorts neurofibrils, Nissl bodies, and mitochondria, but not Golgi apparatus (7). Ultracentrifugation may temporarily inhibit cleavage, perhaps by the creation of large hydrostatic pressures, but effects no detectable separation among whatever functional units are required for development of *Ascaris* eggs (6, 63, 75). Since the same centrifugal forces can separate proteins from solutions, the constituents of cytoplasm are evidently either more coherent than the constituents of protein solutions, or else their spatial relations do not matter in early development.

Micromanipulation continues to be the most adaptable method of deforming cells. A narrow stalk may connect two cells of a dividing sea urchin egg (25). In this thin piece the superficial hyaline layer may be observed to be stiff and elastic, while the interior layer flows and may break up into droplets. Puncturing or cutting a cell just before division gives rise to deformed daughters that make partial readjustments to the typical processes of cell division (25).

Reversible deformations also follow treatment by lack of oxygen and excess of carbon dioxide, and by hypertonic and hypotonic solutions, in the shrinkage of chromosome clumps in the salivary gland cells of fly larvae *in situ* (15, 147).

Moore (110) attempted to estimate the sizes of structural particles in plasmodia (*Physarum*) from the fact that they crawl through filters having average pore diameters down to 1μ . On the other hand, forcible squeezing through pores as large as 200μ in diameter does not allow the slime molds to survive. Commintion by diverse filters alters the living substance so that oxygen is consumed at decreased rates.

FLOW

The fluid state of protoplasm is most extreme in those instances where flow may be observed. Sometimes this takes the form of cyclosis or streaming (100); in other cases it is related to amoeboid movement (91); in others it occurs in special channels that form

during cell division (25) and in churning movements induced in denuded sea urchin eggs (25). Flow of the most superficial layers of eggs during cleavage is studied (42, 43) by placing kaolin particles upon the surface. New cell walls then form by deposition *in situ* without overgrowth, but granules also collect by inward movements near cleavage furrows.

In physical terms the flow of protoplasm is that of a heterogeneous liquid. When it flows it gains a double refraction indicating asymmetry of its particles (121). But its self-propulsion depends on the presence of oxygen (77).

VISCOSITY

This is the most frequently tested of the mechanical properties measured upon living protoplasm; it quantitatively evaluates static and kinetic properties. Some nine distinct procedures are used in viscosity measurements (107) and values are obtained upon diverse protoplasms ranging from two to eight hundred times that of water. This reflects the enormous diversity in fluidity; the more liquid portions of cells are those usually investigated.

The nucleolus, treated as a falling sphere, and the Brownian movement of granules, yield concordant values (ten times that of water) for the viscosity of nucleoplasm in sea urchin eggs (60).

Centrifugation may be criticized as a measure of viscosity alone. The reproducible values obtained by it show recoveries of viscosity after diminution by electric currents (sea urchin eggs) and by temperature rise (*Amoeba*) (1, 2).

When tumor cells are centrifuged at extremely high speeds no displacement of visible contents occurs. This is taken to indicate higher viscosities than in corresponding noncancerous cells of the same tissue mass, which fact may be related to the prevalence of other peculiarities, especially in mitosis (57).

ELASTICITY

Viscosity often decreases with repeated centrifugation. This effect is variously related by investigators to breakdown of structure, to elasticity, and to other factors (81, 83, 114). Further evidence for the presence of elasticity resides in the fact that a lower limit exists to the centrifugal force that will produce any movement at all (114). Killing one *Spirogyra* cell makes neighboring cells in the filament less elastic; from this state they recover during

forty minutes (114). Many other influences (electrical, ionic, thermal, anesthetic) temporarily decrease the elasticity.

Elasticity has also been studied in chromosomes by micromanipulation (4, 14, 46). Large-sized chromosomes are visible in the salivary glands of *Chironomus* and *Sciara* larvae and in the oocytes of *Triturus* and *Rana*; after pulling the threads or the loops even to several times their initial lengths they promptly return to their original lengths and positions.

Cohesion is exhibited most markedly when strands of protoplasm are pulled out from eggs (25). On release the strands spring back to their former places in the rounded mass. The tensile strengths of cohesion presumably correspond to the tensions measured at surfaces. Some tensile strengths of chromosomes were estimated by Duryee (46).

ADHESION

Adhesion is studied particularly in leucocytes in suspension with quartz and carbon particles. In the early work of Fenn, the chances of contact were computed and found to correspond to collisions counted. Local changes in cell surface at points of contact lead to sinking in of the particles. The changes of surface energy during this phagocytosis are indicated by measured rates and depths of penetration of the particles (97).

Adhesion is a property of superficial cell layers. As seen in capillary endothelium (27), the cement substance of the cell boundaries is the material to which carbon particles adhere. When prodded with a microneedle, adhesiveness is greatly enhanced, erythrocytes also sticking to the cement. High calcium and low pH in perfusing fluids also increase the stickiness. It is scarcely appropriate to say that this cement material is nonprotoplasmic; it is believed that the permeability of capillaries depends largely upon it.

The surface changes in *Nereis* eggs at insemination include the formation of filaments that adhere to the vitelline membrane (73, 115). Adhesion of oil droplets and of the sperm, followed by their incorporation, may involve some of the same surfaces (24).

EXTRACELLULAR MATRIX

"Isolated" cells are less common than cells imbedded in cement or matrix. Few kinds of cells in multicellular organisms survive in-

dividual isolation (in tissue cultures), and from this and other considerations it may be inferred that the matrix is an indispensable part of those living systems. In one direction, this phenomenon has been elucidated by explanting cell masses under varying conditions and in different culture media.

More recently attempts have been made to modify the extracellular matrix *in situ*. Duran-Reynals showed that extracts of testis and other tissues when injected into the dermis hastened the spreading of particulate materials. He now finds that dyes pass out of the blood stream faster through capillary walls under its influence (45). The testicular extract has also been tested upon synovial fluid, where it rapidly reduces the viscosity, presumably by virtue of the presence of a mucinase (22). Fractionation indicates the active agent to be protein in nature (22, 98). Similar mucinases are found in leech extracts; they decrease the viscosity of prepared mucoprotein from rabbit skin and extracts of chicken tumor (32). It is believed by some (48, 106) that all "tissue diffusing agents" are mucolytic.

The cement may be studied microscopically in living endothelium (27). It stains differentially with silver nitrate and the color gradually disappears. Perfusion with fluid either free of calcium or high in pH softens the cement and enhances the dissipation of the stain.

MISCIBILITY

Miscibility of protoplasm with aqueous media is studied in ruptured cells. The preservation of cells appears to depend upon their maintaining partially liquid interiors in liquid surroundings. Whether solid is always in between is doubtful; indeed the definitions of solid seem inadequate at this point.

The fatty nature of the envelopes of some cells is indicated by the spreading of caps of oil over their surfaces (79). Similar properties are demonstrated by the interfacial technique of Mudd (112). Under a coverglass, oil fills half the area, a suspension of cells the other half. Gradually the oil displaces the aqueous solution, and as it does so the cells at the boundary are seen to remain wetted to different degrees, as indicated by the distortions of the boundary lines. Several kinds of leucocytes wet with extreme diversity, perhaps representing recent sensitizations and plasma conditions rather than more innate differences in constitution.

When a film of protein covers an oil droplet, minute wrinkles appear in the surface, indicating that the surface is larger than the interface requires and its tension is zero (Devaux effect). Such wrinkling appears in sea urchin eggs only at cytolysis (78). The duration of oil contact before and after cytolysis is a factor. Possibly this is evidence that proteins are not free to precipitate at the cell surface before lysis begins.

What then is cytolysis, in which the mechanical barrier between protoplasm and aqueous surroundings breaks down? Many agents upset the equilibrium between the two so that they mix. Often, however, mixing begins and then recovery follows with the formation of new visible surfaces [the surface precipitation reaction of Heilbrunn (67, p. 79)], and usually the segregation of any included fluid into vacuoles. Numerous influences such as those of ions partially indicate the nature of recovery processes.

SUMMARY

All the above mechanical properties are under current investigation in cells of several varieties. Most can be observed only when the living substance is subjected to suddenly changed conditions, producing a kinetic situation.

Both solid and liquid phases exist in cells. In some, regular transformations of sol to gel, and the reverse, are observed. Studies of changes in viscosity relate these transformations to factors of the environment, and also to flow, division, and death.

The surfaces of cells have limited tensions, perhaps characteristic of differentiated layers. They maintain the integrity of the cells. These or adjacent layers also exhibit adhesion, and are concerned in the maintenance of extracellular matrices that envelop cells and that bind cells together.

ELECTRICAL PROPERTIES

Electrical measurements may be made upon tissue masses, isolated whole cells, unitary surfaces, or particles such as granules. It is often assumed without foundation that any optically homogeneous protoplasm is also electrically homogeneous.

POTENTIAL

Polarization.—The polarization of surfaces of whole cells is indicated by measuring steady potential differences between the in-

side and outside. In isolated giant axons of squid, the concentration potential is not always proportional to the logarithm of outer electrolyte concentration (151). Action potentials as high as 50 mv. are obtained in them from exactly opposed wire electrodes (41). In isolated crab fibers changes of 60 mv. are found (70a).

Some egg cells show permanent potential differences (150), but not sea urchin eggs (129). At fertilization and at cytolysis, transient potentials appear in impaled sea urchin eggs, but only in calcium-free solutions (129). This potential may be concerned with processes that form new protoplasmic surfaces more or less promptly.

Whether the potentials found in bundles or masses of cells are the same as would be found in isolated cells is uncertain. In any measurement of potential the medium is also part of the system under observation, as is well recognized in injury potentials of muscle and nerve. Velocity of conduction is also influenced by media outside an isolated cell (70b).

Electrophoresis.—Electrophoresis allows measurements of zeta potentials in particles of all sizes. These potentials are not expected to equal potentials obtained by placing an electrode inside the same structures. In an imposed field, migration of the particles or cells occurs, and from the rate of migration per unit of current the effective potential may be estimated. Most cells are negatively charged compared to aqueous suspension media; values have been summarized by Heilbrunn (67, p. 87).

Of the visible cell structures, the nucleus of newt eggs moves to the anode; so also the chromosomes and nucleoli (47). When the current ceases to flow they spring back to place. In salivary gland cells of larval *Sciara* the nucleus moves to the cathode, while the chromosome mass moves to the anode (31). Cytoplasmic granules of newt eggs move to the cathode (47); the same holds true for crystals in *Amoeba* and chloroplasts in *Elodea*, both of which may be reversed by adding ammonium salts to the medium (68). This suggests, as in most zeta potentials, a relation of amphoteric electrolytes to the electrical charges.

RESISTANCE

In giant axons of squid it is possible to separate the internal protoplasm from the membrane. The former then has a resistance 1.4 times that of sea water (37), indicating little dilution of the

conducting solution by other more resistant materials. In nerve bundles from other animals (crab, frog) the resistance is precisely proportional, beyond the first 0.8 cm., to the distance between electrodes (44).

In sea urchin eggs a transient change of direct current resistance occurs during fertilization and during cytolysis (129).

IMPEDANCE

Impedance toward alternating currents is ascertained by taking the ratio between the series reactance of the living material and its series resistance, as described by Cole & Curtis (34). At the same time the phase angle of the impeded current is ascertained. Reactance includes the factors of self-induction and of capacity, and varies with the frequency.

Squid giant axons show diminutions of transverse impedance with excitation (36). The greatest change is in the membrane resistance, 97 per cent of which disappears with passage of the impulse. The capacity decreases by 2 per cent, and the phase angle is unmodified. Similar changes occur during conducted activity in *Nitella* (34).

Sea urchin eggs show the same impedance locus whether measured in suspensions (38) or in single eggs (35). Most of the resistance is not at the fertilization membrane but near the plasma membrane, the remainder of the egg being electrically similar to sea water.

Rhythmical impedance changes appear spontaneously in the trout egg some hours after its deposition in water. Both unfertilized and fertilized eggs exhibit the rhythms, with fluctuations of the order of 0.4 per cent, in cycles of about one minute duration. The frequency increases with rise of temperature and ceases with killing (72). The effect disappears reversibly when eggs are treated with phenyl urethane (130), hence like cell division and oxygen consumption (33) can be temporarily inhibited.

SUMMARY

Probably most living cells manifest differences of electrical potential between the inside and the outside. Local differences may exist within them; their prevalence gives rise to hypotheses concerning colloidal aggregation, membranes, and the like. The potential differences are not large enough to furnish considerable

forces. Nothing is known of the rate of production of electricity in subcellular units. The correlation of resistances, reactances, phase angles, and impedances allows neat quantitative descriptions of electrical properties. In trout eggs these properties show a spontaneous rhythm; in cells that conduct impulses they change, suddenly and reversibly, with each excitation.

THERMAL PROPERTIES

Most thermal properties are studied only in large organisms. This is a practical consideration wherever calorimetry is required, as in measurements of heat production, heat loss, and heat capacity. Heat transmission is also unexplored in single units of protoplasm as such. For the most part it is assumed that production, transmission, and capacity are evenly distributed through the substance of a tissue or organism.

Attempts have been made to measure vital functions by the thermal changes involved in dying. The energetics of *Nekrobiose* has been reviewed by Lepeschkin (85). It is hypothesized that heat evolved at killing by means of mercuric chloride or ultraviolet radiation represents a physical decomposition of units called *Vitaids*. In hashed beef liver the heat produced in dying is 0.5 gm. cals. per gm. dry weight (87). Lysis also produces heat. In the death of yeast there may be an absorption of heat instead of an evolution (88).

At temperatures below freezing, most organisms are killed (95). However, survival frequently occurs if freezing is very sudden, the protoplasm being vitrified. In that case recovery depends upon rapid devitrification so that no visible ice crystals form (96). Undoubtedly additional factors are at stake, particularly in those organisms like slime molds that are killed without ice formation (53). The topography of ice crystallization in supercooled organisms also indicates some of the physical differentiations of protoplasm (24).

INFERRED PROPERTIES OF PROTOPLASM

From sheer necessity one research is frequently built about a single method of observation or experimentation. But it is widely hoped that each research contributes information that can be synthesized with other bits that are independently obtained. Up to now efforts at synthesis have been predominantly speculative,

generalization has been by leaps, and concepts have remained broadly untested.

STRUCTURE

The structure of protoplasm in a static sense is the basis for one group of concepts. Visible constitution, represented in the discussions of Just (73) and of Studnička (152), suggests differentiation of function among its parts. Recognizing the diversities among many protoplasms, and the absence of visible differentiation in some, the notion that each structure has a functional significance may not be completely general. However, the contrasts between superficial substance and interior substance, even when they continuously interchange, seem considerable. Division of labor between nucleus and cytoplasm has scarcely been established (11, 12) in other than optical terms.

Macromolecular constitution is being widely discussed, as by Gauze (52), Frey-Wyssling (50), and Seifriz (145). Uniform orientation into fibrous and lamellar configurations are revealed by polarized light. How much permanent structure is present in interior and in flowing protoplasm has not been ascertained. Rapid centrifugation reveals little evidence of permanent displacements that involve function. Such equilibria as exist between built-up ultrastructures and their constituents in solution have only been imagined.

Colloidal constitution is studied mostly by models and analogies (49, 156). Measurements of viscosity and gelation give some quantitative basis to the more general similarities. How much of the colloidal mixtures are arranged in macromolecules and in monolayers is not known.

Molecular constitution is studied in extracted substances (154) and in oriented solids (139). That crystalline forms are present in some materials, emphasizes the probability that arrangement is present at every level of size from atom to cell.

ACTIVITIES OF PROTOPLASM

The activities of protoplasm are manifested in terms of physical changes, and one sort of effort is to correlate these kinetic events in terms of forces and energies. Is the growth of cells, as exemplified in the chitinous sporangiophores of *Phycomyces*, to be described in terms of correlated structures and forces (20)? Can it be

analyzed into movements of discontinuous units and their rates of deposition (21)?

Movements of protoplasm are mostly observed in streaming and in amoeboid progression. What does their slowing under high hydrostatic pressures indicate in the factors of mechanics (100)?

Cell division presents a picture of many simultaneous events. Gelation (99), streaming (25, 26), nuclear migration (23), swelling, contraction, elongation (30), and migration of surface (43) are somehow coordinated in sea urchin eggs. None appears to lead the others, and any one of them may be dispensable in one sort of cell or another.

Conduction of impulses, and excitation, are recognized by physical manifestations. In the squid and the octopus a range of axon diameters with a factor of 24 allowed Pumphrey & Young (127) to establish the fact that axon diameter is directly proportional to velocity of conduction. Excitation by electrical means shows the same characteristics at all sizes (126). The physical changes other than electrical that occur in excited protoplasms, cannot at present be adequately described.

CORRELATIONS

Correlations are most possible among observations on a common species of protoplasm. Many investigators formulate their concepts of protoplasm in terms of what they recall of appearances in *Amoeba*. Actually, considerable exact information exists from which quantitative interrelations among appearances and activities might be made out in that organism.

Recent information is partially available in motile *Amoeba* concerning ratios of gel to sol (24, 101, 103), body volume (105), ingestion of food (104), double refraction in pseudopodia and nuclei (137), and the need of certain granules for locomotion (148); while in spherical *Amoeba* measurements exist of viscosity changes after shaking and after thermal stimulation (2), of tension at the surface of oil droplets within the body (66), and of electrical charges upon granules (68).

Sea urchin eggs have likewise been studied by community of effort. Some years ago Harvey (62) listed the physical and chemical information available concerning them. If every measurement represents some maintenance or functioning of the protoplasm, then those properties that exist together and are modified together

describe a part of its physiological pattern. It is now possible to add a dozen studies of protoplasmic properties of the unfertilized egg (1, 23, 24, 26, 33, 35, 39, 59, 60, 61, 78, 111) half a dozen changes during fertilization and artificial activation (24, 25, 38, 61, 76, 111) and an equal number of specific activities during cell division, as listed above (p. 202).

Squid giant axon has burst into favor as an object of study. One cell contains several milligrams of protoplasm that can be seen, handled, expressed, and analyzed. Optical, mechanical, chemical, and electrical properties have been measured (8, 36, 41, 70b, 126, 127, 140, 151). Which of them are modified in parallel during conduction, isolation, and change of medium may be ascertained. The properties that remain after the cell casing is removed, and the length of time that isolated portions can maintain each function, may indicate much about protoplasm.

Those who are concerned with the specific materials of mammalian physiology and pathology may be particularly interested in leucocytes (91, 97), erythrocytes (9, 51, 93, 112, 113, 125, 146), endothelium (27), cardiac muscle (55), sarcoma cells (25, 57, 92), and extracellular matrix (see p. 195, 196).

Leading hypotheses of protoplasm at the present day deal with structural constitution in terms of macromolecular units, and with phase boundaries as loci of oriented forces. In current concepts structural particularity has displaced randomness, quantitative measurement has succeeded mere detection. While only the static properties have been visualized far, methods are now available for investigation of the multiple aspects of processes that go on. Maintenance and dynamical accomplishment, as simultaneous manifestations of organized protoplasm, form a basis for the current study of cellular physiology.

SUMMARY

The physical properties of protoplasms are known at present in proportion to the availability of methods. Among optical properties, recent advances concern especially polarization optics of living cells and x-ray diffraction patterns of killed material. These have transferred emphasis from the concepts of protoplasm as random colloidal systems to those of oriented and patterned materials. Actually the transformation of ideas results from observations

upon kinds of cellular material other than those that were formerly believed to be representative.

Among mechanical properties, the proportions and arrangements of granules and of gelated materials receive attention. Tension at cell surfaces, formation of cortical layers, and superficial stickiness are being measured. Internally, viscosity and elasticity are the properties susceptible of quantitative evaluation. Organization of cells into masses depends in part upon the variable properties of extracellular cement substances.

Electrical measurements of potential, resistance, and impedance characterize certain localized processes such as cytolysis. Thermal properties are revealed in death changes and in vitrification as a means of surviving low temperatures.

Kinetic processes of protoplasm are illustrated in the visible activities of growth, movement, cell division, conduction, and others. The relations among properties that are modified in connection with each of these activities may serve to describe the nature of these vital events. The exhaustive study of single types of protoplasm such as squid giant axon are suggested as valuable approaches to realizing this objective.

In the past, the various physical techniques have been applied to *Amoeba* and to the sea urchin egg by virtue of their large sizes and isolated situations. Most methods are now applicable to much smaller cells even while imbedded in tissue masses (double refraction, viscosity, adhesion, miscibility). Transitional advances are being made by selecting large representatives of massed cells. Shortly it may be possible to ascertain the relations among events that proceed in one cell or portion of it, to the extent that they are now understood in a whole heart or kidney. Purposes in the study of organs and of cells do not differ; only the techniques differ.

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MUSCLE

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To cover so varied and extensive a field as muscle physiology it is necessary to impose certain limitations upon the subjects to be covered. This review will not consider, therefore, the chemical changes concerned with energy metabolism, the special physiology of heart muscle, histology of muscle, effects of muscular exercise in the intact animal, muscle pathology, or papers appearing previous to about September 1938.

This subject was last treated in this *Review* by Bozler in Volume I. A more recent review of certain aspects of the subject has been published by Buchthal & Lindhard (29). A collection of review articles dealing with various phases of muscle physiology will be found in *Biological Symposia* (14).

Muscles in locomotion.—In recent times little attention has been paid to the mechanical behavior of muscles in the body during walking, running, or other activities. To some extent this involves merely a study of the central motor innervation of the muscles. It is chiefly this aspect of the subject which is considered by Gray (73) with special reference to invertebrate locomotion. The purely mechanical aspects of locomotion have now been analyzed in further detail in the cat by Manter (116) and in man by Elftman (58, 59, 60). The latter study included calculation of the torques exerted in running and walking, the pressure of the foot against the ground and the point of application of this force (60), the function of the arms in walking (58), and the significance of the two-joint muscles. From the forces exerted and the corresponding changes in length the energy expenditure in running was calculated. The limb muscles expend energy at a rate of 2.89 h.p. This agrees closely with a previous value of 2.95 h.p., calculated by a totally different method by Fenn. The distribution of this energy as given by Elftman is 0.15 h.p. against wind resistance, 1.37 h.p. for fluctuations of total energy of the body, and 1.09 h.p. for the distribution of energy between parts of the body (59).

Length-tension diagrams.—Most of the mechanical properties of muscle can be represented by a length-tension diagram. In rest-

ing muscle, according to Brisbin & Allen (25), the increase in length resulting from a given load is proportional to the logarithm of that load. However since, two or sometimes three different proportionality constants are required to fit the curve for a single muscle, this law does not appear to be of much use. In single isolated muscle fibers no single equation, either hyperbolic or exponential, satisfactorily represents the relation in all muscles (132). The function is therefore complex.

Banus & Zetlin (9) have shown that in mammalian muscles the tension developed by extension of the resting muscle is entirely due to the connective tissue. Ramsey & Street (132) have shown further that in isolated frog muscle fiber from which most of the perimysium has been removed the resting tension is due almost entirely to the sarcolemma. The contractile substance itself exerts tension only when stimulated, the tension being maximal at about resting length. Contractions were reproducible without injury to the muscle over a range of lengths from 70 per cent to 200 per cent of the resting length. By continued tetanus the fiber could be made to shorten to 20 per cent of its resting length, but after shortening to less than 70 per cent its properties were irreversibly changed; spontaneous relaxation to its initial length no longer occurred, and the tension developed on subsequent stimulation at any one length was less than before. Some derangement of internal structure evidently occurs when excessive shortening is no longer prevented by connective tissue.

Changes of elasticity have been used (154) to follow the onset of rigor mortis in the mammalian psoas muscle. As is well known, the onset of rigor is hastened by struggling of the animal prior to death, but curiously enough, it is not affected by epinephrine or by brief electrical stimulation of the excised muscle. Decrease of extensibility of frog muscle has been observed during contracture produced either by chloroform or by exposure to 800 atm. pressure (136).

Force and velocity of shortening.—Various attempts have been made to explain the relation between the force exerted by a muscle and the speed with which it is able to shorten or do work against that force. Some investigators have foreseen the possibility of relating this mechanical property of the muscle to its heat production and have urged that the speed of shortening is limited by the rate at which the muscle can develop the extra energy needed for short-

ening. A. V. Hill (90) has now succeeded in formulating a theory of this sort which serves to unite in one equation both the mechanical events and the energy changes of the active muscle, just as the thermodynamic theory of the thermoelastic effect does for resting muscle. Right or wrong, this theory is certain to prove stimulating to the further advance of the subject. He has in the first place fitted the force-velocity (pv) curve by the equation for a rectangular hyperbola by adjusting the asymptotes. Thus

$$(p+a)(v+b)=b(p_0+a)$$

where a and b are constants and p_0 is the isometric tension. With a suitable choice of values for a and b the equation fits the data reasonably well, but this cannot be regarded by itself as a very critical test of the correctness of the theory. If, however, the quantity ab is subtracted from both sides, the equation may be written

$$v(p+a)=b(p_0-p)$$

Since a has the dimensions of force, it may be regarded as the internal resistance to shortening so that the total resistance $(p+a)v$ is the rate of energy liberation. The equation states, therefore, that the rate of energy liberation is proportional to p_0-p , i.e., to the difference between the isometric and the observed tension. The equation in this form is found to fit the observed rates of heat production obtained from thermopile measurements, using the same values for the constants a and b as were derived for the force-velocity data. This equation means that muscle viscosity is really a matter of the rate of energy liberation for shortening, as some have long maintained. Using the same equation with some subsidiary assumptions Hill (90) has calculated a theoretical isometric tension-time curve which gives an approximate fit to the experimental data. The theoretical relation between speed and efficiency also agrees with the experimental results (88).

The equation should fit data obtained from stretching the muscle at varying rates, but Katz (101) has shown that other constants are required. In tortoise muscle the same equation applies to velocities and heat production during shortening, but the tension-time curve shows large divergencies (101). A further application of the equation has been made by modifying it for the case of a human arm muscle shortening against an inertia wheel at different velocities (91). A good agreement was found between the theory

and the old experimental data of Hill & Lupton (see also 27, 148, 149).

Muscle and rubber.—Several authors in seeking a clearer insight into the cause of muscular contraction have been impressed by the similarity in the physical properties of rubber (when slightly stretched) and muscle. Both substances are peculiar in that they show a high degree of extensibility with a small coefficient of elasticity; both shorten when warmed and give off heat when stretched; both show double refraction, shorten under pressure (51) and decrease in volume on shortening (Ernst). Wöhlisch (174, 175, 176, 177) has formulated the thermodynamic relations between these various properties (cf. also 76). He has stressed particularly the statistical-kinetic theory as opposed to the interatomic attraction theory in explanation of the phenomena. According to this theory the elastic force is represented by the tendency of the oriented molecules to return to a more random distribution by thermal agitation. The heat given off on stretching is an orientation heat rather than a heat of crystallization. One of the predictions of this theory is that the muscle force at constant length should vary with the absolute temperature, as it does in rubber.

The tendency of muscle to shorten when warmed has been re-investigated in both heart muscle (177) and in fascia-free skeletal muscle (137). In both cases the linear thermal extension coefficient was found to be negative, in agreement with earlier work, except when it was stretched so much that the positive coefficient of the connective tissue became dominant. According to Wöhlisch the contraction of muscle which results from exposure to high pressure is the physical consequence of its structure and the orientation of its molecules. Stretched rubber shows a similar effect. Photographic records of the pressure contraction of muscle have shown, however, that the contraction lags behind the rise of pressure and gives a latent period of 0.03 seconds before the contraction begins. On this account it has been concluded that the contraction is not a purely physical shortening but a true physiological response (83).

Following further the similarity between rubber and muscle, Ebbecke (52) has made a model of striated muscle by overstretching alternate sections of a thin rubber band and can demonstrate, for example, a decrease in double refraction on heating. In the light of this model he discusses the structure and the contraction of muscle. The viscous-elastic properties of unvulcanized rubber have

also provided a close parallel to the mechanical properties of smooth muscle as exemplified in the foot of the snail (113) and the body wall of the sea anemone (104).

Oxygen consumption.—By following the time course of the oxygen consumed by frog muscle after stimulation at low temperatures, where the rate of diffusion does not limit the supply of oxygen, it has been shown that oxygen is not needed or consumed until after the contraction even if it is available (87). Oxygen, therefore, is not used directly for contraction but only for recovery. The regularity with which the recovery heat equals the initial heat under a wide variety of conditions, i.e., twitches and steady states, also testifies to the reality of the heat as a recovery phenomenon and not merely as a temporarily increased resting rate (89).

An increased consumption of oxygen by the smooth muscle of *Mytilus* in tonic contractions has been demonstrated (134). Measurements of the anaerobic oxygen debt of frog tissues indicate that muscle is unique in its ability to contract and to pay off an oxygen debt (141). An important study by Stannard (159) indicates that the excess oxygen consumed by muscle in activity may be specifically inhibited by sodium azide. Thus the oxidation systems involved in activity differ from those at rest.

Lindhard (108) concludes that a muscle contracting with no load liberates no extra heat and consumes no extra oxygen. His experiment consisted of applying fifteen "short" tetanic stimuli to the nerve of a frog gastrocnemius muscle. Comparison was made between the isometric contraction and a contraction with one end of the muscle free. Heat was measured only qualitatively, but the excess oxygen used was approximately ten times greater in the isometric experiment. Since tetani were used the experiment was largely a comparison between isometric contractions at two different lengths; and since the diagonal-fibered gastrocnemius was used, the "isometric" condition at the greater length was complicated by considerable internal work which greatly increased the heat production. The result was, therefore, entirely in accord with expectations.

Excitability.—In agreement with previous work, three different types of muscle excitation curves have been described (20), together with the changes which they undergo with nicotine. This change is chiefly a curarization signaled by the disappearance of the type of curve due to nerve. In order to study muscle excitabi-

lity, therefore, it is necessary to arrange the experiment in such a way that only the alpha or the true muscle curve is obtained.

Because of the small degree of accommodation which it shows, frog muscle can be made to respond repetitively with fifty or more twitches to alpha excitation by a constant current only slightly stronger than rheobase (18). The frequency of such repetitive discharges in curarized sartorius muscles stimulated by constant currents, is greater with a greater strength of stimulus (106, 130), and it is diminished 30 per cent by the addition of vitamin C (147). As the duration of a rectangular stimulating current increases, the height of contraction increases in steps, each step representing the summation of a further contraction from a newly initiated impulse. A similar result was reported for stimulation of the frog muscle through its nerve (49). In a summation of a few contractions of this sort, the magnitude of the summated height depends upon the interval between the two stimuli, there being, of course, a certain optimal value which occurs usually when the second contraction begins just at the peak of the first. Thus it may happen that a short weak galvanic stimulus may initiate the same number of impulses as a shorter stronger one, but the weaker current may cause a greater summated contraction because the interval between the responses is longer and more nearly optimal (122). Alpha stimulation of isolated fibers may initiate a response at any point of the fiber without regard to the position of the degenerated end plate (133). This may be taken as synonymous with the finding (13) that the physiological properties of the muscle under the end plate do not differ from those found elsewhere on the fiber. The responses to alpha stimulation may be of the usual all-or-none propagated variety or they may be local twitches graded in magnitude in proportion to the strength of the stimulus. The strength-duration curve is the same for both types (133). In the single muscle fiber the graded nonpropagated contraction is said to be unaccompanied by a refractory period (150).

It seems probable that this local contraction is a normal phenomenon. According to Feng (62) and his collaborators, Wedensky inhibition brought on by high frequency nerve stimulation is accompanied by a nonpropagated contraction and a local negativity under the end plate. A similar end-plate potential and a local potential due to incompletely propagated "new born" impulses under

the end plate have been described by Eccles & O'Connor (54). A local end-plate potential was also reported by Schaefer (144).

Of theoretical importance for the excitation of muscle is the measurement of the electrotonic change under the stimulating electrode (145). This local potential is probably the outward sign of the local excitatory process and may also be represented by the end-plate potential in the myoneural junction (144). When the electrotonic potential builds up to a value of about 3 m.v., a propagated response is observed to start. The rate of decay of this potential is similar to the rate of decay of the local (α) excitatory process. The latter decays more rapidly after short subliminal stimuli than after longer ones (10, 102).

When a constant current applied to muscle is interrupted by pauses of sufficient duration and frequency a tetanus results. The boundary conditions of frequency, duration of pauses, and strength of current necessary to cause such a tetanus have been systematically defined (96).

Of interest from the point of view of the myoneural junction is the effect of acetylcholine on the α excitability of muscle. No effect is observed (17) until the concentration becomes high enough to cause fibrillary twitching at which time the rheobase is lowered and the chronaxie is prolonged. This would suggest that acetylcholine cannot function to facilitate an electrical stimulation. Others have concluded, however, from similarities in behavior that acetylcholine and electrical stimuli act upon the same rather than different mechanisms (11). Furthermore by intra-arterial injection of acetylcholine a transitory decrease in threshold for electrical stimulation was observed which lasted about as long as the contracture produced by the drug. The change in excitability also occurred even when the concentration was too low to cause contracture, and was not eliminated by the previous denervation of the muscle (100). The end plate is particularly sensitive to acetylcholine, potassium chloride, and the electric current as was shown by microapplication of these stimuli to the end plates of the isolated single fibers of the lizard (30) (cf. also 12). Application of acetylcholine to the end plate may thus cause one response, but this renders the end plate insensitive to the nerve impulse and to a second application of the same substance. An excess of acetylcholine may therefore cause a block. Bathing the surface of a fatigued

frog muscle with acetylcholine (or nicotine) has been found to improve the contraction (129). Fatigue of muscle is usually due to failure of myoneural transmission or to failure of excitation. In the latter case recovery from fatigue induced by direct electrical stimulation is accelerated by anodal and diminished by cathodal polarization with a galvanic current (181).

Little further progress has been made in the understanding of the refractory period of muscle but mention should perhaps be made of the attempt of Umrath (169) to distinguish between an autogenic or intrinsic refractory period of short duration and a longer one induced by outside influences.

Resting potential.—A thorough study has been recently reported by Sugi (162) of the distribution of potential along a toad sartorius muscle and in the Ringer's solution surrounding the muscle. The positivity on the surface of the muscle is especially high near the cut end, and if both ends are cut, the positivity in between is greater the shorter the length of intact muscle. This, however, only represents the distribution of the electric field, all of which can be explained by postulating a polarized cap or dipole layer at the cut surface. The author concludes that his results are inconsistent with the membrane theory which postulates that all the polarization is uniformly distributed on the intact surface. Actually it would seem to be impossible to distinguish between this condition and the single polarized cap by measurements of the type reported.

Other similar potential measurements indicate characteristic gradients along the intact surfaces of frog muscles after slight injury by treatment for 5 to 10 sec. with mercuric chloride (163). The distribution of potential around the freshly cut surface of a gastrocnemius muscle was also carefully measured. It is doubtful in this case also whether the author was correct in interpreting his results as contrary to the usual membrane theory. The negative potentials of freshly injured surfaces decay rapidly with time and the rate of decay is said to be accelerated by selective stimulation of adrenergic fibers to the soleus and by cholinergic fibers to the gastrocnemius (123). An explanation of the effects of various organic electrolytes and nonelectrolytes on the resting potential has also been offered (95).

Action potential.—This subject has been recently treated in this *Review* (75) so that little further comment is required. Of particular interest is the discovery of local end-plate potentials,

already mentioned, which may be taken as evidence in favor of a predominantly electrical transmission at the myoneural junction. In isolated single muscle fibers Wilska & Varjoranta (172, 173) have reported a shower of impulses at an initial high frequency of 70 to 300 per sec. as a result of piercing the fiber with a micro-electrode. These impulses travel in both directions at a rate of 1 to 2 m. per sec. increasing with increase of temperature. This proves the existence of propagated action potentials in muscle which are independent of end plates. On stretching the fiber 50 per cent there is only a 30 to 35 per cent increase in conduction time or in other words a 10 per cent increase in conduction rate.

By flexing the human arm against a special mechanical interruptor which serves to synchronize the impulses recorded in the electromyogram, it has been possible to estimate the increase in the number of impulses which accompanies an increase in the intensity of contraction (156). In the muscles used in the abduction of the finger the frequency of the electrical changes for a standard contraction against a load of 20 gm. was diminished by a previous contraction against 900 gm. (99). This would seem to represent the same phenomenon as the familiar decrease in frequency or increased synchronization of impulses with advancing fatigue. The electromyogram of the frog gastrocnemius during a reflex contraction was found to be devoid of any component which could be attributed to the sympathetic fibers (48).

The frequency of the sound waves recorded from muscles is supposed to be related to the frequency of the innervating impulses (21) and simultaneous sound and electrical records from pigeon muscles in strychnine tetanus have demonstrated similar frequencies in the two records (24). In the complex sounds recorded from a whole contracting human muscle, however, no increase in pitch could be detected. A very thorough history of the subject of muscle sounds is given by Bouman & van Rijnberk (21).

Myoneural transmission.—The theory of myoneural transmission is still so imperfectly developed that it is impossible to give a rational account of the appalling array of disorganized facts which are so rapidly accumulating in this field. The electrical theory still has its adherents. The familiar inhibition which occurs with high frequency indirect stimulation is attributed to some sort of depolarization of membranes which is maintained by incoming impulses (180), and the localized end-plate potentials in the myo-

neural region (54, 144) are regarded as the electrical sign of the intermediary link between nerve and muscle. The facilitating effect of a nerve impulse at the myoneural junction can add to the facilitating effect of a short cathodal electric pulse and both effects subside at the same rate. This suggests that the effect of a nerve impulse is an electrical depolarization of the membrane (102). The histological problems of interpretation of the end plate in terms of electrical theory have been outlined by Blair & Street (16). It is generally admitted that the end plate acts like a synapse and it has been suggested that it is therefore homologous with the postganglionic fibers of the autonomic system (55).

On the other hand, the somewhat more elastic chemical theory continues to score successes (34) and it has been applied particularly to explain the series of alternating increases and decreases in the mechanogram of a muscle during continuous indirect tetanus at various frequencies. According to Rosenblueth & Cannon (140), seven different phases can now be recognized. After the initial rise there are two successive inhibitory depressions each followed by a rise of tension, and after these five "early" stages there is a prolonged phase of transmission fatigue in which the formation of acetylcholine by the nerve endings is supposed to be low. It is certain that this is not a purely muscular fatigue because it appears equally well if the muscle is prevented by temporary curarization from contracting at all during the early stages of stimulation (112). If stimulation is continued in spite of this fatigue, there is a final slow recovery of tension and action current (139) during which the acetylcholine content of the nerves also increases from the previous low fatigue level (138). At low frequency stimulation only the first stage and these last two appear. With higher frequency the early stages of depression appear (Wedensky inhibition). A similar temporary depression has been described in partially curarized and in magnesium-treated muscles (22). At least one of these early inhibitions has been explained as due to an excess of acetylcholine but no explanation for both of them has been offered as yet (35).

A post-tetanic enhancement of the twitch has often been attributed to a mobilization of potassium. Since the distribution of potassium in the muscle is influenced by adrenalectomy it is interesting to find that adrenalectomized animals fail to show the usual post-tetanic potentiation (15). In contrast to some previous results the potentiation has been found absent in denervated or curarized

muscle (74) and after potassium treatment. This post-tetanic enhancement is absent in muscle during transmission fatigue (35). A somewhat similar phenomenon results if one branch of the sciatic is stimulated rhythmically at 30 stimuli per min. while the other branch is stimulated with a short tetanus. After tetanus of some of the fibers of the muscle, the twitches of the other fibers are increased in height (118).

Eserine tends to prolong the end-plate potential and to induce repetitive responses to single or especially to double nerve volleys (64, 71) and may even lead to spontaneous impulses (53). These rhythmic responses lead to fascicular rather than fibrillar twitching in the muscle which is accompanied in the motor nerves by antidromic impulses (119) which are abolished by curare. The effects of eserine are usually interpreted in terms of its action on acetylcholine esterase (72) but this is not always regarded as necessary (53) nor adequate. In an attempt to utilize practically the potentiating effect of eserine, injection of the drug was found to increase the work capacity and to decrease the lactic acid level during activity in both rats and men (124).

The persistent contracture of the forearm muscles of frogs, which results from rhythmical stimulation of the motor nerve, is particularly evident in males and is favored by physostigmine and by potassium (23).

Veratrine has been shown to produce its characteristic prolongation of the twitch by causing a repetitive response (56, 98, 126). With large doses the oscillatory action potentials tend to disappear (63), which probably explains the failure of Inoue to observe them (98). Thus if only one end of a muscle is poisoned with veratrine the typical contraction is produced at both ends because the extra impulses are normally conducted. According to Bacq (5, 8), the action of veratrine is explained by its ability to sensitize the muscle to the action of potassium ions, as well as rubidium, cesium, ammonium, and barium ions. In the presence of veratrine only one third to one fourth as much potassium chloride is required to produce the same physiological effects (7). Using veratrine it was possible to demonstrate the humoral transmission of excitation by potassium ions liberated from one frog muscle on stimulation and carried in the perfusion fluid to another frog (6).

The veratrine contracture has been likened to myotonia (82), and like that condition of persistent contraction it is abolished by

quinine. The effect of quinine in preventing the development of tetanus from a single volley at the myoneural junction is attributed to a lengthening of the refractory period and to a decrease in sensitivity to acetylcholine (81). Its effect upon the muscle twitch itself is to slow the contraction and thus to increase the tension developed. Quinine thus has a curare-like effect and abolishes the repetitive response caused by eserine (64).

Other cases of neuromuscular abnormality which have been recently investigated are the effects of tetanus toxin and of calcium deficient diets. Tetanus toxin produces a local tetanus which is purely peripheral in origin and is dependent upon the presence of end plates. The fibers are spontaneously and irregularly active and the nerve endings respond to a single nerve volley with a diminished but repetitive response (80). In fowl muscle a single nerve volley fails to elicit a maximal response but succeeding volleys at sufficient frequency induce responses which increase progressively to normal values. Lack of dietary calcium (26) intensifies this condition in fowls and may cause it to appear in goats.

The concentration of end plates in a muscle determines the concentration of cholinesterase. For this reason the activity of this enzyme is high in embryonic chick muscle (128). In denervated muscle the activity persists unchanged even when the nerve endings have degenerated; this shows that the activity is in the end plate rather than in the nerve endings proper (45). Of particular interest also is the fact that this enzyme is found in especially high concentration in the electric organs of *Torpedo*; these are regarded as an accumulation of cholinergic end plates (61, 127).

Electrolyte distribution in muscle.—It has been argued that all the chloride in muscle is in the connective tissue or extracellular spaces. About 13 per cent of the muscle volume in frogs is accounted for in this way. In support of this, Sandow (142) has calculated a space of 14.5 per cent if the cylindrical fibers are so packed that half are arranged in squares and half in hexagons. Maurer (120) has succeeded in extracting a minute sample of extracellular fluid from a frog muscle and has found that its chloride and protein content resembles in accordance with theory an ultrafiltrate of plasma. Chao (37) found that the chloride space gave an accurate measure of the space into which hemoglobin and indigo carmine would diffuse. In trying a similar experiment with phenol red Maurer (121) found that this dye apparently diffused into 35

per cent of the muscle when the chloride had diffused into only 27.7 per cent. He showed, however, that this difference was due to a concentration of dye in the "dead" cells (which are responsible for the increase in the chloride space on soaking in Ringer's solution) rather than to an actual penetration into live cells. Evidence that the chloride space is not merely extracellular space but includes various other fractions has also accumulated. Thus, during a period of soaking in Ringer's solution the chloride space increases by steps (69) as more and more parts of the muscle become permeable to chloride. Glucose behaves similarly but penetrates always a slightly smaller fraction of the muscle than the chloride. Other similar experiments have been interpreted to indicate that only 9 per cent of the muscle is immediately permeable to chloride and sodium and is therefore truly extracellular space (43). The diffusion of inulin is also confined to 9 per cent of the muscle. On immersion in glucose the sodium and chloride of this space are lost quickly, but the remainder emerges much more slowly. Sodium is regarded (43) as present in the sarcoplasm together with some chloride, while the potassium is considered to be confined to myofibrils. A direct attack upon the problem was attempted (47) by analyzing single isolated frog muscle fibers for chloride by an ultramicro method. The small amount of chloride found was no greater than could be accounted for as contamination from the Ringer's solution which had not been washed off by the preliminary technique. In rat muscles the chloride space amounts to 40 per cent at fifteen days of age and decreases to 15 per cent in adult rats at ninety days of age (93). In *Mytilus* smooth muscle the sodium space is increased from 30 per cent to 60 per cent by soaking in saline solution. Glucose, sodium, barium, and potassium diffuse freely into this enlarged sodium space (151).

That there is no bound water in muscle is indicated by the fact that ethylene glycol diffuses freely into all the muscle water (97). When muscles are immersed in solutions of different osmotic pressures, however, the weight changes of the fibers (assumed to be chloride-free) are such as to indicate that 15 per cent of the water is osmotically inactive (67b). This was confirmed by the data of Chao, Chiao & Chi (39) when properly calculated (37). The result possibly indicates that all the chloride is not strictly extracellular under all conditions. Wolf (178) has noted that frog muscles immersed in Ringer's solution first gain and then lose water. The

magnitude of the changes is somewhat modified by previous hydration of the frog, but the phenomenon is not readily explained purely on the basis of osmotic changes.

The electrolyte pattern in muscles of the squid (115) and the sea cucumber (160) has been studied and found to differ only in detail from the patterns of vertebrate muscle. In both species the ratio of sodium to chloride is the same in muscle as it is in the surrounding fluid (blood or sea water), the chloride space being 16 per cent in the squid and 40 per cent in the sea cucumber. There is a large cation deficit in the squid muscle. Potassium can be made to move into the cells of the sea cucumber against a concentration gradient if the outside potassium concentration is increased. Values for electrolyte concentrations in dolphin muscles have been reported (57). The extracellular space in this species has an unusually low value of 8.5 per cent.

As a result of another histochemical study of muscle (131) it is concluded that potassium is uniformly distributed throughout the fiber, a result in much better accord with purely chemical data than the highly localized distribution previously reported by this method. The calcium and magnesium of muscle, on the other hand, have been clearly located in the contraction bands by the use of the electron microscope (146), the tissue spaces being practically free of these elements.

Further study of the potassium loss from muscles during contraction indicates that the amount lost is greater in isometric than in isotonic contractions; it is, in general, proportional to the magnitude of the contraction but is especially increased by rhythmical rather than continuous contractions (65). The loss of potassium is greater in young than in adult rats, is decreased by tenotomy and is not affected by low potassium diets or iodoacetic acid poisoning (85, 86).

In cats the loss of potassium on stimulation is decreased somewhat by previous injection of calcium chloride or cortin and is slightly increased by potassium chloride (164). On the other hand, Somogyi (158, 171) found no loss of potassium on stimulation of cat muscles after bilateral adrenalectomy.

The loss of potassium on stimulation is completely eliminated by curare (67, 179); this shows that the loss is caused by the contractile process, although it has been claimed that the excitatory

process causes an increased solubility of potassium (without actual loss) which is not eliminated by curare (135).

A prompt increase in the concentration of potassium in the venous blood from stimulated muscle has been observed in dogs (179), cats (67, 158), and frogs (67). In dogs the prompt return of potassium to the muscle in recovery has also been followed by analyzing venous and arterial bloods (179). The rise of potassium in the venous blood begins within ten seconds at least of the time of stimulation and is followed after a slight delay by a rise in the lactic acid content (67).

Along with the familiar electrolyte changes in contraction there is a loss of histamine from preformed stores. This is true both in skeletal (2) and in smooth (1) muscles. An increased concentration of cholinesterase in the venous blood from stimulated muscle has also been reported (44).

Smooth muscle of *Mytilus* loses water and a proportional amount of base when it is stretched; like striated muscle, it gains sodium on stimulation (151).

The loss of potassium from cat muscles during asphyxia, caused by diminution of blood flow, was found to be very small (66). In frogs perfusion of the hind legs with oxygen-free solutions caused no detectable loss of potassium (67a). Isolated frog muscles immersed in oxygen-free solutions lost little more potassium than similar muscles in oxygen, although the loss became appreciable when glycolysis was also prevented by iodoacetate poisoning (47). Lowry reported that potassium is lost more slowly from isolated rat muscle immersed in oxygen-free Ringer's solution than from a heated control muscle (110). These experiments are of interest in connection with the idea that the retention of potassium in muscles is dependent upon the continuation of oxidative processes.

Of importance for the theory of electrolyte equilibrium in muscle is the finding of Steinbach (161) that frog muscle immersed in solutions of high potassium content not only gains potassium but also loses sodium. This is most marked after previous immersion in solutions of low potassium content, in which case the reverse change is observed. Likewise Heppel (86) has raised rats on low potassium diets (cf. also 125) and finds half the muscle potassium replaced by sodium. This sodium is certainly inside the fibers to a large extent but exchanges freely with injected radioactive sodium

(85). The muscle appears, therefore, to be able to retain potassium in spite of the fact that it is permeable to sodium.

A new theory of electrolyte equilibrium in muscle has been proposed briefly by Conway & Boyle (42), according to which the muscle is permeable to chloride and other monovalent anions, but the chloride concentration inside should be only as great as the potassium concentration outside. A small concentration of chloride of this magnitude inside the cell would be difficult to exclude experimentally.

Effects of electrolytes.—Magnesium increases the chronaxie and rheobase of frog muscle (111). When injected into dogs (114) it decreases the height of contraction, inhibits the response to acetylcholine and is antagonized by eserine. Magnesium, strontium, and barium can replace calcium in Ringer's solution (38), but barium has a secondary excitatory effect. Barium and calcium decrease the electrical resistances of frog muscle while magnesium, potassium, and sodium have little effect (78).

Heilbrunn (84) has observed that muscle fibers, like some other types of protoplasm, contract when immersed in pure isotonic calcium chloride solutions. From this rather drastic experiment and other indirect evidence it is proposed that a mobilization of calcium is the cause of a normal muscle contraction. A perceptible liberation of calcium from isolated muscle fibers stimulated to contract by ultraviolet light was detected in 9 out of 33 experiments (4) using alizarin as indicator.

In cats the potassium content of muscle and plasma was varied by low potassium diet, adrenalectomy, and sodium depletion. No correlation was found between the potassium concentration and the magnitude of the contraction (125).

The effects of potassium acetylcholine, and other drugs and electrolytes on smooth muscle of frogs and mammals have been studied further but the additional facts would appear to leave the subject no less confused theoretically than it has always been (152, 153).

An increase of potassium concentration has been found to increase the rheobase and to decrease the sensitivity of the muscle to acetylcholine (41). An increase of sodium chloride or absence of calcium chloride increases rheobase without affecting the sensitivity to acetylcholine (41). Lack of calcium (as well as addition of

guanidine or veratrine) sensitizes a muscle to chemical stimulation by either potassium or acetylcholine (79).

The effects of potassium, sodium, and calcium on rheobase and on k (the coefficient of decay of the alpha excitatory process) are complex and not easily interpreted. While the membrane potential frequently changes parallel to the rheobase, the excitability proper, i.e., the values of k , shows no such simple relationship (36).

The effects of digitoxin on stimulated muscle have been shown to be due chiefly to the potassium liberated from the muscle fibers and accumulating in the extracellular spaces. There is, however, an additional effect in decreasing the ratio of tension to heat on stimulation which is not present when potassium is added without digitoxin (77).

Atrophy.—An excellent review of the atrophy of denervated muscle was recently published by Tower (167), in which consideration was given to the view that the atrophy was the result of exhaustion from the continuous fibrillation which develops. Atrophy from tenotomy where there is no fibrillation may be the result of exhaustion from the contracture which occurs (168). That these explanations are perhaps not well founded is suggested by the finding that fibrillation of denervated rat muscle (as indicated by action potentials) may be prevented by the administration of quinidine without appreciably diminishing the amount of atrophy (155). The action potentials of these fibrillary twitches in the dog's tongue do not disappear on cutting the sensory or the parasympathetic nerves (157).

Data have been presented concerning the loss of weight of dog muscle due to atrophy from denervation, tenotomy, and immobilization (94). In a careful comparison of denervation and tenotomy as a cause of atrophy (105) it has been shown that the muscle force calculated per unit cross section of the chloride-free phase of atrophic muscle shows no change from normal in denervation but a marked decrease in tenotomy. It is suggested, therefore, that maintenance of the initial tension is an important factor in preventing the loss of strength.

The rate of atrophy in monkeys is not affected by massage, passive movements, or electrical stimulation, but the restoration of the muscle after nerve regeneration is said to be helped by physical therapy (40). In rats, also, electrical stimulation has been found

ineffective in retarding atrophy (92) as measured by the loss of weight. This negative result may have been due to the use of stimulating currents of insufficient strength, for with strong currents Fischer (68) found some effects on excitability and weight loss. The loss in birefringence with advancing atrophy was not affected by stimulation, however, and the muscle power was not improved.

As atrophy proceeds in a denervated muscle there is a loss of potassium to which has been attributed the correspondingly increased sensitivity to acetylcholine and nicotine (107). The final failure of neuromuscular transmission has been attributed to a decreasing output of acetylcholine from the degenerating nerves which parallels the decreasing amount of acetylcholine which the nerves contain (109). Mention may also be made of the decrease in the concentration of cholinesterase which parallels the atrophy of muscle in dogs. There is no change in cholinesterase, however, after section of sympathetic nerves only (44, 165). After denervation, the sensory elements in the muscle also degenerate, although more slowly than the motor nerves. Diminution of sensory discharge is also observed after section of either anterior or posterior spinal roots (103).

Other features of the contraction.—The latent period of the toad sartorius muscle has been subjected to further study (143, 166) and a value of 5.8 to 6.1 m. per sec. was arrived at for a temperature of 16°C. after corrections had been applied for errors due to such factors as load, inertia, magnification, and speed of the drum. The result does not differ significantly from the best figures previously reported in the literature.

Margaria & Fornaroli (117) have concluded that the staircase effect in muscle is due to an alkalization of the muscle because it is absent in an alkaline perfusing fluid, and at a maximum in an acid solution and in a muscle poisoned with iodoacetate.

An increase in the magnitude of contraction as a result of sympathetic stimulation or epinephrine has been described in the dog (31). Epinephrine injection had an effect similar to that of sympathetic stimulation and was found to be effective even when the blood flow was simultaneously diminished. Other vasoconstrictor drugs had a similar effect. Since the effect largely disappeared when the muscle was curarized it was concluded that the effect was on the neuromuscular transmission (33). A production of heat in

frog muscle as a result of purely sympathetic stimulation and with no corresponding motor response has been reported (3).

Further measurements of the transparency of muscle reveal rapid changes during contraction (19). In single isolated muscle fibers contraction results in increased dispersion and decreased transmission (28).

Summary.—The last two years has been a time of particular activity in the investigation of (a) the precise relation between the factors of length, heat, and tension, (b) the mechanism of myoneural transmission of excitation, and (c) electrolyte distributions and permeabilities in resting and active muscle.

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THE DIGESTIVE SYSTEM

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Hunger and appetite.—Richter & Barelare (1) made a further study of the fact that rats on a vitamin-B-free diet eat more olive oil and less sucrose and casein than control rats. They found that after addition of riboflavin to the diet, the animals exhibited an increased appetite for casein. Animals receiving thiamin ate normal amounts of sucrose, ceased to ingest olive oil, but refused to eat casein. Nicotinic acid failed to influence the appetite. Rats given the W factor showed a constant and normal olive oil intake. Rats receiving thiamin and riboflavin or all four components of the B complex had an appetite similar to that of control animals, but the most striking effect was the return of the appetite for casein. The authors report also (2) that parathyroidectomized rats exhibited an increased appetite for calcium solutions (lactate, acetate, gluconate) and an aversion toward dibasic sodium phosphate. Those given access to calcium solutions had a lower mortality and less severe symptoms. Rats given access to alcohol solutions and distilled water were apparently unable to distinguish between water and dilute alcohol up to 1.4 per cent alcohol (3). They preferred the alcohol solution to water between 1.4 per cent and 6 per cent but refused to take higher concentrations of alcohol.

Nash (4) observed that 50 per cent of his rats on a starvation diet which were injured either in the tail or the forepaws, ate the injured member up to 2 inches of tail and the entire forelimb up to the elbow. No such autophagia was observed in wounded rats that were normally fed or in unwounded rats even though starved.

Gastric motility.—In 1928 L. G. Cole (5) proposed the surprising hypothesis that gastric peristalsis is a function of the muscularis mucosae and does not involve the outer muscular walls of the stomach. The experiments of Gordon & Singleton (6) suggest that he may have been correct, at least with regard to one type of peristalsis. They sewed various opaque objects such as steel beads to the outer gastric wall in dogs, and then observed the stomachs fluoroscopically after a barium meal. They report that "peristaltic

waves passed distally without disturbing the alignment of the beads," except when the waves were more vigorous than usual. Changes in the positions of the beads were observed which they interpret as tone changes. They think there may be two types of peristalsis, one involving the entire musculature and another involving the muscularis mucosae only.

Quigley, Werle & Brody (7) correlated their studies of intragastric and intraduodenal pressures, by means of optical manometers, with simultaneous fluoroscopic observations. They state that pyloric evacuation and bulbar filling begin without significant antral or bulbar pressure changes. Antral pressure begins to rise two or three seconds later and at or before its maximum evacuation ceases and bulbar pressure rises sharply. Bulbar waves are related to the antral waves in frequency and magnitude. Their observations ascribe gastric evacuation to the moderate basal pressure gradient from antral to bulbar region, to which the sphincter and bulb offer little resistance. Resistance to propulsion due to closure of the sphincter occurs shortly prior to the end of the antral contraction and serves to prevent duodenal regurgitation.

Werle, Brody & Quigley (8) applied the optical manometric method to a study of the effect of gastric filling on antral and bulbar pressure patterns. Feeding 100 cc. of strained corn meal mush by mouth caused an increased pressure in the antrum and bulb after a delay of from one to five minutes. When the material was introduced into the stomach through a gastric fistula the same pressure changes occurred immediately, in both normal and vagotomized dogs.

Painter, Todd & Kuenzel (9) report a study of the motility of the pylorus and duodenal cap by means of serial radiograms; the work is too extensive for adequate review. Perhaps their most remarkable observation is that the pyloric rhythm seems to be independent of gastric peristalsis but is closely co-ordinated with the rhythm of the duodenal cap. This observation would appear to be in conflict with those of Cole, Wheelon & Thomas, Thomas & Crider, Quigley and others, who agree that the pyloric rhythm is co-ordinated with gastric peristalsis [but see Quigley's observations on antral and bulbar rhythm which are cited above (7)].

Gastric emptying time was prolonged by lowering the intragastric temperature but unaffected by increasing it, in the experiments of Eberhard (10). According to Gershon-Cohen, Shay &

Fels (11), lowering the intragastric temperature hastens initial emptying while elevation of the temperature retards initial emptying. Therapeutic doses of hydrochloric acid had no statistically significant effect on the emptying time of the normal human stomach in the experiments of Van Liere & Sleeth (12). Therapeutic doses of sodium bicarbonate, on the other hand, hastened the emptying, on the average by 16.3 per cent. A similar effect (17.1 per cent) was produced by disodium phosphate, thus ruling out carbon dioxide as a causative factor.

Winfield (13) found that in dogs bile or bile salts in the stomach caused an increase in gastric motility if the stomach was quiet but effected a moderate and temporary inhibition if gastric motility was already present.

In experiments described by Tudoranu *et al.* (14), filling the duodenum with distilled water, spinach extract, peptone, glycerin, magnesium sulfate, ethyl alcohol (concentration not stated), or carbonated water inhibited gastric contractions. The last two agents caused a primary increase in motility. Administered rectally, normal saline and carbonic acid solution caused an initial increase in gastric motility followed by inhibition; 4 per cent magnesium sulfate and 3 per cent sodium bicarbonate led to complete or nearly complete inhibition; 10 per cent alcohol increased gastric tonus without affecting amplitude of contraction (15). Intravenous administration of 100 cc. of 33 per cent glucose depressed gastric tone and motility; smaller doses had no effect (16). Necheles, Olson & Morris (17) observed that extreme degrees of hypoglycemia (less than 30 mg. glucose per 100 cc. of blood) caused a decrease in gastric motility in dogs. In moderate hypoglycemia, increased motility occurred.

According to Morrison & Feldman (18) experimental hyperthyroidism, induced by feeding desiccated thyroid gland to dogs, causes an increase in gastrointestinal motility whether or not the vagus nerves are intact. Thyroid medication apparently overcomes the gastric retention which results from bilateral section of the vagi. Removal of the normal thyroid gland causes a loss of tone throughout the gastrointestinal tract only when myxedema occurs.

Artificial fever caused relaxation of the cardiac sphincter and the sphincter of Oddi [Doubilet and Bierman (19)]. Myers (20) reports that morphine, diacetylmorphine, codeine, dihydromorphine, dihydrocodeine, and dihydro-oxycodone increased the

tone, and the amplitude of rhythmic contractions, of the pyloric sphincter in the cat.

Gastric secretion.—The production of carbonic acid from carbon dioxide and water in the presence of carbonic anhydrase may be a necessary step in the secretion of hydrochloric acid by the stomach, according to Davenport (21, 22) who finds that carbonic anhydrase is present in the parietal cells of the gastric mucosa of cats, rats, and dogs. In cats the concentration is five to six times greater than in the red blood cells, in rats about two to three times as great. Thiocyanate inhibits equally the ability of carbonic anhydrase to catalyze the hydration and dehydration of carbon dioxide, and the secretion of acid by the gastric mucosa of dogs (23). The author concludes that "The rate of secretion of acid by the gastric mucosa is directly proportional to the rate of formation of carbonic acid in the secretory mechanism."

Teorell (24) attempted to determine the "primary" acidity of the gastric juice as secreted, before dilution or neutralization. He filled the stomach with a buffer solution and measured the increase in volume and the amount of standard alkali necessary to restore the original pH of the material after it had absorbed the gastric secretion. This method gave the high average value, expressed as hydrochloric acid, of $0.208 \pm 0.006N$. The "primary" acidity was relatively independent of the rate of secretion. He interprets his results as favorable to the Pavlov theory of constancy of the primary acidity of the gastric juice.

The acidity of the gastric juice was found by Taylor & Michael (25) to be unaffected by changes in the carbon dioxide content of the blood plasma. By oral administration of ammonium chloride they reduced the carbon dioxide content of the plasma of Pavlov pouch dogs to 26 volumes per cent, without decreasing the free acid of the pouch secretion. Lyall & Nicol (26) also found the acidity to be unaffected by the hypochloremia and alkalosis brought about by continuous removal of the gastric contents.

The gastric secretion is generally believed to diminish with advancing age; however, Bloomfield (27) found a significant decline in only one of five relatively normal subjects over a ten to eleven year period, but in a study of 75 essentially normal people of different ages (20 to 80 years) he observed that the basal acid secretion was lower in the older subjects. Meyer, Spier & Neuwelt (28) demonstrated a decline in the fasting concentrations of practi-

cally all digestive enzymes except lipase in the various digestive secretions, with advancing age.

Pickett & Van Liere (29) found that low oxygen pressure caused a decrease in gastric secretion in animals provided with Pavlov or Heidenhain pouches. The depression began to be effective at about 80 mm. of mercury pressure and became progressively more severe as the pressure was lowered. The secretion was more severely depressed in the Heidenhain pouches than in the Pavlov pouches.

Inhibition of gastric secretion by means of acid placed in the stomach was investigated further by Wilhelmj & Sachs (30), who found that addition of acid (0.102*N*) to test meals caused a two-thirds reduction in the amount of acid secreted by the human stomach; 0.058 normal acid was without effect. According to Shay, *et al.* (31), 1 per cent hydrochloric acid in the stomach completely inhibits gastric secretion but 0.5 per cent does so only partially. Instillation of 0.4 per cent hydrochloric acid or human gastric juice into the duodenum of normal human subjects or ulcer patients failed to decrease the secretion of gastric hydrochloric acid in the experiments of Stevens, Segal & Scott (32).

Grindlay (33) found that in dogs the removal of the pyloric portion of the stomach failed to decrease the secretory response of Heidenhain pouches to a meat meal. When the antrum was separated from the body of the stomach but remained connected to the duodenum or drained to the outside, the secretion was prolonged following a meal (twelve hours as compared to nine hours in the normal).

Shay, Gershon-Cohen & Fels (34) confirmed the common finding that olive oil in the duodenum causes a depression of gastric secretion followed by stimulation. Apparently the secondary stimulation is not due to soap because sodium oleate and 40 per cent glucose caused both initial depression and secondary stimulation.

Mann & Mann (35) caused transitory achlorhydria to histamine in dogs by exposing the gastric mucosa to dilute solutions of mercuric or cupric salts or to quinone. Salts of lead, manganese, and zinc, as well as hydroquinone and resorcinol were ineffective. Brilliant green and crystal violet stained the gastric mucosa and inhibited acid secretion.

An extensive study of the effects of various antacids on gastric acidity was made by Kirsner & Palmer (36). In general their work

tends to show that most of the commercial antacids available at present are comparatively ineffective in reducing gastric acidity. The most effective combination tested was atropine with calcium carbonate. Komarov & Krueger (37) observed a decrease in gastric secretion upon the administration of colloidal aluminum hydroxide to dogs. The effect was evident in the secretion of Pavlov pouches even when the reagent was placed in the stomach. When colloidal aluminum hydroxide was mixed *in vitro* with canine gastric juice in quantities sufficient to buffer its free hydrochloric acid, it also removed all the pepsin (38). A similar antipeptic action was observed when the reagent was introduced into the stomach. The preparation was useful for controlling skin erosions around the stomata of Pavlov pouches.

Gastric secretion was reported to be suppressed by the barbiturates in anesthetic doses (39) and increased by the administration of vitamin B₁ (40) and by nicotinic acid (41). The latter had a histaminelike effect on gastric secretion in normal subjects but no effect in achylia. Vitamin C was reported to be effective in achylia (42).

Urogastrone, a gastro-inhibitory substance present in normal urine, was the subject of numerous investigations. The early preparations caused fever but Gray and his co-workers (43, 44) devised a method for separating it from the pyrogenic substance. That urogastrone may be excreted enterogastrone was indicated by the experiments of Culmer, Gray, Adkison & Ivy (45), who demonstrated an increase in urogastrone in the urine of dogs fed a high fat diet as compared to fasted controls, and an almost complete absence of the substance after removal of the small intestine. They regard their results as suggestive but not conclusive [see also Ivy (46)]. On the other hand, Friedman, Saltzstein & Farbman (47) demonstrated the continued presence of urogastrone in extracts of the urine of dogs after removal of the stomach or of the duodenum. They argue from these observations that the substance is probably not of gastric or intestinal origin. Their results are also inconclusive inasmuch as enterogastrone may be produced by the upper jejunum as well as by the duodenum.

Wieczorowski, Gray & Ivy (48) administered pyrogen-free urogastrone subcutaneously to nine human subjects. It reduced the basal secretion, and the response to histamine in regard to both the volume and acidity of the gastric juice and the total quantity of

acid secreted. The output of free acid was reduced by 40 to 95 per cent. Except for a little local soreness at the sight of the injection, no undesirable effects were observed. Sandweiss & Friedman (49) made a preliminary report on the apparently successful treatment of twenty ulcer patients with a commercial preparation of urogastrone. Necheles, Hanke & Fantl (50) prepared a urine extract by fractional precipitation with ammonium sulfate and alcohol which depressed or abolished both gastric secretion and motility. Impure preparations also contracted the gallbladder and increased the secretion of bile. Friedman, Recknagel & Patterson (51) tried various means, unsuccessfully, to offset the inhibitory effect of urogastrone on gastric secretion. The intravenous administration of glucose caused an increase in the rate of gastric secretion which was prevented by the previous administration of urogastrone.

Brunschwig, Clarke *et al.* (52, 53) continued their investigation of the inhibitory effect on gastric secretion of neutralized gastric juice. They now find that 78 per cent of samples of gastric juice from patients with gastric cancer and 20 per cent of samples from normal subjects inhibit gastric secretion in dogs when injected intravenously.

Histamine may be responsible for the increase in gastric secretion which follows the administration of alcohol. Dragstedt, Gray *et al.* (54) demonstrated an increase in the histamine output of the perfused lung of the guinea pig when 2 to 6 per cent alcohol was added to the perfusion fluid. Furthermore, Gray & Bachrach (55) found that the secretory activity produced by alcohol resembles that caused by histamine in being resistant to the inhibitory effects of atropine and olive oil. In chronic alcoholics Seymour, Spies & Payne (56) demonstrated diminished response of the stomach to histamine.

Whether histamine increases the output of pepsin or only the secretion of hydrochloric acid remains an open question. Bucher (57), investigating the pepsin concentration of gastric juice secreted by a pouch of the entire stomach following vagotomy, found that small constant doses of histamine given every ten minutes for five to eight hours produced continuous secretion, in which the hourly output of pepsin was constant. She concludes that histamine produces a significant and prolonged increase in the total output of pepsin. On the contrary, according to Pratt (58), if the stomach is washed out beforehand and the pylorus suitably oc-

cluded, injection of histamine causes secretion of gastric juice entirely free from peptic activity.

Ivy & Bachrach (59) attribute to histamine liberated in the inflamed or ulcerated intestine the "hypercontinuous" secretion of gastric juice in Pavlov pouch dogs having Mann-Williamson operations. This interpretation was supported by the fact that atropine depressed the secretion in these animals after a meal to only about the same extent as it did the secretory response to histamine.

Roth & Gabrielson (60) reported that in 95 per cent of swimmers immersion of the body in cold water between 65° F. and 85° F. caused a definite increase in gastric acidity, which appeared fifteen minutes after removal of the subject from the water. Water at body temperature had little effect. Immersion of one hand in water at 10° C. caused a slight rise in gastric acidity. Histaminase introduced by means of a duodenal tube thirty minutes before immersion prevented the effect.

In regard to the nervous phase of gastric secretion, Katzenbogen, Loucks & Gantt (61) failed to obtain conditioning of the secretory response of the stomach to histamine. "Psychic" secretion of gastric juice was demonstrated in human subjects by Bloomfield (62), who found that by merely discussing with patients their favorite foods and the methods of preparing them their fasting gastric secretion was increased in twelve out of fourteen cases. Babkin, Komarov & Komarov (63) found that raising the blood calcium by means of irradiated ergosterol, parathormone, or intravenous calcium lactate reduced the nervous phase of gastric secretion by nearly 50 per cent. Acidity and total pepsin were also reduced. Histamine response was not affected. Van Liere, Vaughan & Northup (64) observed inhibition of gastric secretion in dogs exposed to 100 decibels of noise at a frequency of 2,000 cycles per second. The same amount of noise at a frequency of 600 cycles generally had no effect.

Tudoranu *et al.* (65) found that rectally administered sodium chloride, magnesium sulfate, and bouillon stimulated secretion of acid by the stomach, whereas glucose depressed it. Olive oil (not absorbed by rectal mucosa) and sodium bicarbonate had no effect.

According to Pratt (58), the intravenous injection of secretin excites secretion of pepsin by the gastric mucosa. The smallest dose of secretin which would activate the pancreas was effective. The introduction of bile salts into the duodenum had a similar effect.

Neuwelt, Olson & Necheles (66) described a new procedure for making a Pavlov pouch. It differs from the standard operation mainly in that the initial incision is made through the anterior gastric wall, sparing the muscle layers and nerve trunks of the greater curvature. They claim that their operation preserves the major part of the vagus nerve supply.

Permeability of the gastric mucosa.—Teorell (67) found that the mucosa of the cat's stomach was permeable to weak and strong acids and to various neutral salts, e.g., potassium bromide, sodium bicarbonate, sodium acetate, sodium sulfate, etc. It was relatively impermeable to sodium iodate and to glucose and glycine. He believes that regulation of gastric acidity can be fully explained by absorption of excess hydrochloric acid through the gastric mucosa. On the other hand, Shay *et al.* (31) failed to observe absorption of hydrochloric acid from the stomachs of human subjects in concentrations up to 1 per cent, over periods of time up to thirty minutes. According to Martini, Morando & Minuto (68), sucrose is absorbed in small amounts (20 to 56 mgm. in 30 min.) from the stomach from 10 or 20 per cent solutions. The absorption of glucose in concentrated solution (15 per cent) from the stomach was again demonstrated (69).

Davenport (70) fed sodium bromide to dogs, cats, and rats until 50 per cent of the serum chloride was replaced by bromide. Under these conditions the gastric juice contained hydrobromic and hydrochloric acids in proportions corresponding to the ratio of serum bromide to serum chloride. Brunschwig & Schmitz (71) studied the rate of passage of radioactive chloride from blood into the gastric juice. Within 60 to 120 seconds, and for more than 130 minutes after intravenous injection, they were able to detect radioactive ions in the gastric secretion.

Intestinal motility.—Douglas & Mann (72) studied intestinal motility with a new type of preparation consisting of an exteriorized loop of intestine, in continuity, covered with a tube of skin. Their results are in agreement with the current conception of the gradient theory and our knowledge of the constancy of the rate of rhythmic activity for any given segment. In their experiments the rate of contraction was not affected by fasting, feeding, or sleep, nor by degenerative section of the vagus and splanchnic nerves. They (73) observed an increase in motility in the ileum after giving food, which failed to occur after severance of the intestinal wall, al-

though the extrinsic nerve supply was intact. Double vagotomy did not abolish the response and it was as constant when the animal was fed through a fistula as when the food was taken by mouth. The response to feeding appeared (74) earlier in the jejunum than in the ileum. Section and re-anastomosis of the intestine in such a way as to prevent immediate union of the muscular coats and the intrinsic enteric plexus did not prevent the occurrence of the response to feeding below the point of section. The response was also seen in the distal part of the colon but not in its proximal part.

In two patients with prolapse of the caecum following caecostomy, the ileocaecal musculature was found to be relaxed most of the time (75). Inserting the finger into the caecum or colon induced contraction of the sphincter. Epinephrine caused relaxation, acetylcholine, momentary relaxation followed by increased tone. Activity increased after meals. A study of the motility of the terminal ileum by x-ray cinematography revealed rapid propulsive and slow churning movements but no contractions of the ileocolic sphincter (76).

Yeast contains a substance, soluble in 50 per cent alcohol, which accelerates the passage of food ("Purina" dog chow) through the intestine of dogs, according to Russell & Nasset (7). The substance apparently is not vitamin A nor D nor any known member of the B complex.

Elsom & Drossner (78), using the technique of intestinal intubation as developed by Abbott and others, observed a decrease in tone and in peristaltic activity of the human small intestine following administration of therapeutic doses of atropine sulfate and tincture of belladonna. Rhythmical contractions were also diminished but to a lesser degree. They (79) demonstrated an increase in motor activity of the entire intestinal tract following administration of pitressin. Amphetamine (benzedrine) sulfate exerted a sympathomimetic effect in that tone and peristaltic activity were decreased. According to Gruber, Haury & Drake (80):

Apomorphine when injected intravenously may either increase or decrease the general tonus of the intact intestine, depending upon the animal and the condition of the animal at the time of the injection. The peristaltic contractions may be augmented by apomorphine, especially when the general tonus is diminished.

In the experiments of Myers, (81) all the opium alkaloids tried increased tone and peristalsis in the small intestine (cat) and in-

creased the tone of the ileocolic sphincter. Atropine antagonized morphine. Epinephrine caused temporary inhibition after morphine.

Lawson & Chumley (82) found that intralumen pressures higher than 30 mm. of mercury in loops of ileum in barbitalized dogs caused a significant decrease in blood flow through the intestinal vessels which became progressively greater as the pressure was increased.

Bussabarger (83) described a method for the study of intestinal function in surviving dogs. The preparation consists essentially of a Thiry-Vella intestinal loop which is exteriorized and protected by a layer of skin.

Intestinal secretion.—Wright, Jennings, Florey & Lium (84) studied the effect of vagus and splanchnic section and stimulation, and of certain drugs and hormones, on intestinal secretion. They found that stimulation of the vagus nerves, in decerebrate or decapitate cats, caused secretion from the duodenum which came chiefly from the Brunner gland area but none from the jejunum or ileum. Cutting the greater splanchnic nerve in the thorax caused secretion from the duodenum only. Cutting all the preganglionic sympathetic fibers caused secretion from all the small intestine. (Efforts to exclude the effect of vascular changes failed.) The subcutaneous administration of eserine caused secretion from all the small intestine. They confirmed the presence of a secretion hormone (not proved to be other than secretin) acting on the first part of the duodenum in cats. It was shown to be present also in pigs (denervated loop method) and probably in dogs (innervated loop). The duodenal secretions and secretions from other levels of the small intestine of five different species, from denervated and innervated fistulae, were alike in that they contained amylase, enterokinase, and traces of invertase and lipase, but no protease or peptidase.

Fogelson & Bachrach (85) observed secretion of a viscid juice from Brunner's glands of the duodenum following intravenous injection of vasodilatin-free secretin but they were uncertain whether this secretion was independent of the increased motility of the duodenum which resulted from the injection.

Schiffirin & Nasset (86) reported that feeding causes diminution of enzyme concentration and total enzyme secretion of jejunal and ileal segments of dogs, lasting for from six to eight hours.

Denervation reversed this effect. Feeding or denervation augmented the response to enterocrinin, which increased the concentration and quantity of enzyme. Enterocrinin in large doses was effective subcutaneously, differing in this respect from secretin.

Northup & Van Liere (87) found that the secretory activity of the intestinal mucosa was highly resistant to anoxia, showing only a moderate decrease at 53 mm. of mercury oxygen pressure, corresponding to an altitude of 28,000 feet.

Herrin (88) demonstrated secretion of ammonia by the small intestine of the dog in amounts mainly related to the amount of nitrogenous material in the food.

Intestinal absorption.—Visscher & Roepke (89) reported further on their observation that sodium chloride is absorbed against a concentration and osmotic pressure gradient when isosmotic mixtures of sodium chloride and sodium sulfate are placed in the small intestine. Poisoning the intestine with mercuric chloride decreased absorption of chloride and increased absorption of sulfate and tended to abolish the gradients. The studies were extended to unanesthetized dogs by Dennis & Visscher (90) with similar results. Absorption was not affected by feeding but was slowed by excitement. Lifson (91) studied the phenomenon in rats and rabbits; maximal blood-gut chloride concentration ratios of 10 to 1 in rats and 3 to 1 in rabbits were obtained, as compared to ratios as high as 200 to 1 reported in dogs. Van Liere & Vaughan (92) found that absorption of chloride against a diffusion gradient was not greatly reduced by anoxia although some slight depression of absorption was observed.

Dennis & Wood (93) demonstrated a marked decrease in the rate of absorption of sodium, potassium, and chloride from the ileum of adrenalectomized dogs after withdrawal of adrenal cortical hormone. The rate of sodium absorption declined, in general, more than that of potassium, leading even to a reversal of the direction of movement of sodium. The decrease in osmotic activity of fluids placed in the intestine which is seen in normal animals was less rapid after withdrawal of adrenal cortical hormone. Minibeck (94) found that the selective absorption of glucose and galactose (these sugars are absorbed three to four times more quickly than fructose, mannose, sorbose, or xylose) from the duodenum of big Hungarian frogs was abolished by adrenalectomy or by hypophysectomy (anterior lobe), all sugars being absorbed at the rate

characteristic of fructose, etc. Davidson & Garry (95) report:

In cats anesthetized with urethane and 'Pernoctin' xylose is absorbed as rapidly as glucose from the caudal region of the small intestine. In this respect the cat differs from the rat. Galactose appears to be absorbed more rapidly and fructose more slowly than glucose.

Roberts & Westenhoeffer (96) found that 0.435 per cent phlorizin prevented absorption of glucose (2 per cent solution) from the small intestine. Lactoflavin had no effect on the phenomenon. Lium & Florey (97) find that isotonic magnesium sulfate slows absorption of sodium chloride but not of glucose in the cat. Sodium chloride is best absorbed from the ileum, glucose from the jejunum. The cathartic action of isotonic magnesium sulfate was thought to be due to its effect in slowing absorption. Iodoacetic acid or potassium cyanide, according to Given (98), failed to block absorption of fluorescing dyestuffs as observed in transparent crustacea with a fluorescence microscope.

Shay, Gershon-Cohen *et al.* (69) failed to confirm Cori's view that there is a constant rate of absorption of glucose per unit of time independent of the concentration. In their experiments absorption from the duodenum was greater the higher the concentration of glucose, above 15 per cent.

Coffey, Mann & Bollman (99) studied the utilization of food-stuffs by normal and experimental dogs by means of analysis of the feces. When bile was excluded from the intestine (100), there was a definite decrease in absorption of fat, but carbohydrate utilization was not impaired. In experimental pancreatic deficiency (101), total pancreatectomy, or evulsion of the pancreatic ducts there was a marked loss of fat and carbohydrate in the feces. Following evulsion of the ducts only, the loss developed gradually and appeared to parallel the degenerative changes which occurred in the acinar tissue of the pancreas. They (102) were able to restore normal carbohydrate utilization (absorption) by administration of pancreatic juice, raw pancreas or pancreatin preparations, in very large amounts, but were unable to bring about normal utilization of fat by any replacement therapy. Even continuous instillation of pancreatic juice into the upper jejunum failed to restore normal fat utilization.

Crandall & Ivy (103) found that sodium dehydrocholate was less efficient than commercial bile salts in restoring normal fat absorption in bile fistula dogs. Carotene is not absorbed from iso-

lated intestinal loops in the absence of bile and pancreatic lipase, according to Irvin & Kopala (104). Both bile and lipase are necessary for maximal absorption. Apparently bile is also necessary for absorption of adequate amounts of fat-soluble vitamin K (105). Absorption of indole from the jejunum in healthy rats (106) and of water suspensions of cholesterol (without fat) from the alimentary tract of rabbits (107) was demonstrated.

Young, Phillips & Murlin (108) demonstrated a moderate amount of absorption of insulin from the gastrointestinal tract when insulin was protected from digestion by addition of certain alkyl resorcinols. Best results were obtained with hexylresorcinol in alkaline solution (pH 9.9 to 10.5).

Moore, Arrowsmith, Welch, & Minnich (109) studied absorption of iron compounds from the gastrointestinal tract by means of determination of "serum iron" increases after administration of iron compounds by mouth. Their report contains much valuable information of which there is space here for only the following: Only ionized iron is absorbed and that in the ferrous state. Gastric acidity has little or no effect on absorption of easily ionized ferrous compounds. Reducing agents greatly increase absorption when ferric iron is given and also aid in absorption of ferrous iron.

Freeman & Johnson (110) investigated further the hemolytic effect of lymph collected from lacteals and the thoracic duct during fat absorption. The hemolysis was attributed to fatty acids and soaps, which were found in the lymph in quantities ranging from 1 to 5 mg. per cc. They suggest

that during rapid absorption of the products of fat digestion, complete resynthesis to neutral fat particles does not occur, leaving free fatty acids or soaps in sufficient amounts to be hemolytic.

Colon.—Regan & Puesto (111) studied the motility of externalized isolated segments of the dog's colon by direct inspection without anesthesia. Their observations confirm the earlier findings of Templeton & Lawson that there are three types of motility of the dog's colon: (a) small waves caused by contraction of the circular muscle, not propulsive in nature; (b) true peristalsis; and (c) mass contractions involving the circular and longitudinal musculature.

Templeton & Adler (112) described a technique by which the propulsive force developed in the colon can be correlated with the

motility occurring at the same time. The greatest force was exerted on an immovable object in the colon during the first half of the activity period, the least during the last quarter. They also found (113) that the greatest rate of propulsion occurred during the first quarter of the active period. The proximal colon showed a more rapid rate of transport than did the distal colon. Morphine greatly increased the propulsive activity of the dog's colon in experiments by Adler, Krasno & Ivy (114). Atropine antagonized the effect of morphine on propulsive activity but did not significantly reduce the nonpropulsive activity of the circular muscle.

Ivy & Goldman (115) report that distention of the colon (proximal part especially) reflexly inhibits secretion of bile by the liver. Stimulation of the colonic nerves or the inferior mesenteric plexus caused an increase in resistance to flow of fluid from the bile ducts into the duodenum due to contraction of the sphincter choledochus (Oddi).

Pancreas.—The secretagogue action of water on the pancreas, which is evident when distilled water is injected into the duodenum, is not exhibited by various isotonic solutions, including isotonic sodium chloride and 5 per cent glucose, according to Crider & Thomas (116).

Marked changes in blood sugar level were without effect on the secretory volume or proteolytic activity of pancreatic juice obtained from unanesthetized dogs in the experiments of Scott, Bugel, and co-workers (117). The spontaneous secretion was also independent of the blood sugar level and was irregular in onset and duration and not always associated with periods of hunger activity (118). However, hunger activity was almost invariably accompanied by pancreatic secretion, which might occur during any part of the period.

In the experiments of La Barre & Kettenmeyer (119), generalized hyperthermia or circulation of the blood of a hyperthermic dog through the head of another inhibited the response of the pancreas to secretin (in the latter case, in the recipient). Naselski (120) found that the concentrations in the pancreas of potassium, calcium, magnesium, and chloride, total and inorganic nitrogen, lactic acid, non-protein nitrogen, and amino nitrogen were all decreased following secretin injection, whereas glycogen was increased. During activity the pancreatic blood lost glycogen and gained lactic acid.

Best, Haist & Ridout (121) observed that daily administration of insulin, fasting, or feeding diets rich in fat, caused a decrease in the insulin content of the pancreas; insulin administration produced the greatest effect. The insulin content was restored to a normal value within six days by means of a well-balanced diet. The fall in insulin content and recovery on a balanced diet occurred also in hypophysectomized rats (122). The authors conclude that the Beta cells of the islands of Langerhans are "rested" by fasting, insulin, and by fat feeding.

In experiments described by Popper & Necheles (123), the concentration of amylase and lipase increased in the portal blood and in the lymph following ligation of the pancreatic ducts. The authors concluded that the bulk of the enzyme was absorbed by way of the blood stream. Golden and others (124) found that permanent separation of the pancreas from the duodenum resulted in a persistent increase in the activity of diastase in the blood which was present even when the pancreas had atrophied to a small mass.

Important advances in experimental methods applicable to the pancreas were made during the year. Diamond and co-workers (125) used a relatively pure preparation of secretin obtained from Astra in Sweden for the study of pancreatic function in human subjects. Boldyreff & Thompson (126) and Crider & Thomas (116) devised new methods for collecting pancreatic secretion which permit the pancreatic juice to flow freely into the intestine between observations. McCaughan (127) described a new type of pancreatic fistula made by exteriorizing the uncinate process of the pancreas after ligation of the pancreatic ducts. When the exposed portion is cut off the secretion escapes in a retrograde manner through the severed duct.

Lipocaic.—A factor capable of preventing excessive deposits of fat in the livers of depancreatized dogs is present in the external secretion of the pancreas, according to Montgomery, Entenman, Gibbs & Chaikoff (128). They fed pancreatic juice to depancreatized dogs for a period of twenty weeks. In contrast with untreated dogs, the treated animals did not show excessive fatty infiltration of the liver. Depancreatized dogs fed with whole meat powder from which the extractives had been removed also failed to develop fatty livers in the experiments of Ralli & Rubin (129, 130). After addition of meat extractives to the diet of these animals, fatty infiltration of the liver occurred as usual. In depancreatized rats,

meat powder failed to prevent development of fatty livers, whereas the extractives had only a slight lipotropic effect.

Rubin & Ralli (131) observed that the accumulation of fat in the livers of depancreatized and pancreatic-duct-ligated dogs was accompanied by a marked fall in the blood plasma lipids. Similar results were obtained by Dragstedt and his co-workers (132), who observed, however, that the lipids rose somewhat above normal during the first week after the operation and then gradually declined. The oral administration of lipocaic corrected this hypolipemia within a week.

Julian, Clark & Dragstedt (133) observed that the fatty infiltration of the liver which is produced by administration of the ketogenic hormone of the pituitary gland was prevented by lipocaic but not by choline. The fatty livers which develop in scorbutic guinea pigs were not influenced by lipocaic nor by choline (134), and lipocaic failed to prevent arteriosclerosis and hypercholesterolemia in rabbits fed on cholesterol dissolved in sunflower seed oil (135). Clark, Walsh, Julian & Dragstedt (136) treated twelve cases of psoriasis with "lipocaic" for periods of from five to fourteen months with favorable results.

The history and present status of lipocaic have been reviewed by Dragstedt, Vermeulen, and others (137) and by Dragstedt (138), who discussed its relation to diabetes in the human. He suggests that disorders of fat metabolism and the tendency to arteriosclerosis of diabetic patients may be due to lipocaic deficiency.

Innervation.—Wang, Clark, Dey & Ranson (139) studied the effects on the gastrointestinal tract of stimulating various parts of the hypothalamus. Stimulation anterior to the infundibular region caused immediate blanching and occasional inhibition, followed by a marked excitatory response of gradual onset and several minutes' duration. Division of both vagus nerves did not abolish this effect. Since it failed to occur regularly in chronic spinal cats, the authors hesitate to attribute it to stimulation of the pituitary. Typical vagal effects on the intestine resulted from stimulation of the hypothalamus at or behind the infundibular level. These responses were present in spinal cats and were abolished after vagotomy.

Burstein (140) demonstrated in dogs a marked increase in intestinal motility during spinal anesthesia which persisted for at least thirty minutes after sensory and motor recovery. In this connection it is interesting to note that Telford & Simmons (141) report

permanent relief of seven cases of megacolon and one case of mega-esophagus (cardiospasm) by means of spinal anesthesia.

Grondahl & Haney (142) succeeded in causing a condition which they considered analogous to clinical cardiospasm by combining thoracic vagotomy with a girdling incision through the muscular coats of the esophagus at a point about 4 cm. above the diaphragm.

Drake, Modern, Renshaw & Thienes (143) failed to demonstrate sensitization of the muscle of the excised intestine to acetylcholine, pilocarpine, eserine, nicotine, barium chloride, or potassium chloride following degenerative section of the vagi, splanchnics, or both. Splanchnic degeneration sensitized slightly to epinephrine. After section of mesenteric nerves the intestinal and vascular muscles were sensitized to epinephrine.

Pfaffmann (144) demonstrated the presence of nerve impulses in the dental nerves during stimulation of the teeth by means of touch or pressure. Thresholds for the canine teeth were in the neighborhood of 2 or 3 grams of pressure with a bristle. Vibratory stimuli applied to the teeth caused rhythmic impulses in the dental nerves which corresponded in frequency to the frequency of the vibration, up to about 1500 cycles (145). He also demonstrated nerve impulses in the chorda tympani and glossopharyngeal nerves of the cat following application of sapid substances to the tongue (146).

Kanaev (147) noted marked individual variations in salivation in response to unconditioned stimuli which were stable for two to three years in different individuals but a close correlation between unconditioned and conditioned responses in the same individual (148).

Experimental peptic ulcer.—The increasing list of substances by means of which ulcerative lesions of the gastrointestinal mucosa may be produced experimentally received the following additions: histamine (149), acetylcholine (150), various aliphatic amines (151), epinephrine (152), and aspirin (153). The histamine was mixed with beeswax and injected intramuscularly into cats, in which it caused a continuous secretion of acid gastric juice, and produced gastrointestinal lesions consisting of erosions of the mucosa or acute ulcers or both. The acetylcholine was given to dogs by constant injection of small amounts; the lesion was described as a hemorrhagic condition of the stomach and duodenum;

stimulation of the vagus, centrally, had a like effect. The aliphatic amines such as methylamine, ethylamine, propylamine and isobutylamine in concentrations above 1/15 molar produced acute gastric erosions or ulcer when applied subcutaneously; intoxication of the liver by chloroform or phosphorus aggravated the effect. Intraperitoneal injections of epinephrine in dogs, cats, rabbits, and guinea pigs produced acute ulcerative lesions of the gastric and intestinal mucosa; in rabbits and guinea pigs the lesions were, pathologically, identical to lesions found at autopsy in human patients after surgical shock. Oral or subcutaneous administration of aspirin or oral administration of acetic and salicylic acids produced gastric ulcers in mice and rats; mixing sodium bicarbonate with the aspirin prevented the ulcers. Berg (154) observed that frequent injections of pitressin caused lesions of the stomach in a higher percentage of vagotomized animals than of normal dogs. Vagotomy without pitressin did not cause ulcers.

Slive, Bachrach, & Folgerson (155) attempted to produce jejunal ulcers in dogs by means of the Mann-Williamson operation modified so as to obtain a better nutritive state of the animal. Twelve animals that lost weight developed ulcers but eleven animals that gained weight did not. The authors suggest that malnutrition is a factor in the etiology of Mann-Williamson ulcers. Florey, Jennings, Jennings & O'Connor (156) attempted to produce intestinal ulcers by the Mann-Williamson technique and by various similar operations in pigs. In general, their results indicate that the portion of the intestine supplied with Brunner's glands (duodenum and jejunum in the pig) is resistant to ulceration caused by contact with unneutralized gastric juice. In pigs they were able to cause typical ulcers only in the ileum. They had previously demonstrated a greater resistance to the action of gastric juice in the duodenal mucosa of the dog as compared to the mucosa of the ileum or jejunum, which they attributed to the Brunner's glands in the duodenum.

Neuwelt & Necheles (157) found that the volume and acidity of the gastric secretion produced by histamine in Heidenhain pouch dogs was not increased during the administration of cinchophen. They noted also that white Spitz dogs seem more prone than other breeds to develop cinchophen ulcers.

Slutzky & Wilhelmj (158) report that the administration of phenolphthalein is not a satisfactory test for the presence of ulcer-

ation in the gastrointestinal tract of experimental animals. Some of their ulcer animals failed to give positive reactions, whereas jaundiced animals gave false positives.

Experimental intestinal obstruction.—Fine, Fuchs & Mark (159) found that the marked fall in plasma volume observed in dogs subjected to continuous distention of the small intestine was at least partly prevented by the intravenous administration of desoxycorticosterone. However, Jorgensen, Dietz, & Hill (160) could not detect a significant rise in the plasma potassium level of dogs with intestinal obstruction, or with a segment of intestine deprived of its blood supply, or a combination of these conditions. In acute obstruction in dogs produced by ligature of the duodenum, Guruchaga (161) found that although normal dogs collapsed and died in two to three days, dogs from which the splanchnics and abdominal sympathetic chains or the nervous plexus surrounding the celiac trunk and superior mesenteric artery had been removed three to four weeks previously, did not collapse acutely and lived five to six days.

Popper & Necheles (162) studied the effect of mixtures of gall-bladder bile and pancreatic juice on the occluded small intestine. No damage occurred to the intestine but there was evidence of diffusion of pancreatic enzymes through the intestinal wall into the peritoneal cavity.

General.—Whether the duodenal mucosa elaborates a hormone which aids in the control of carbohydrate metabolism was seriously questioned by Loew, Gray & Ivy (163), who were unable to demonstrate any effect on blood sugar of acid stimulation of the duodenum. They (164) state also that carefully prepared extracts of the intestinal mucosa, free from pancreatic tissue, obtained by a number of methods which have been reported to yield a hypoglycemic substance, consistently failed to affect the blood sugar level of fasted, unanesthetized dogs. Nevertheless, Rothschild & Berger (165) contend that an extract which is not insulin and does not affect the islands of Langerhans but which does affect blood sugar may be obtained from gastric and intestinal mucosa. The substance does not alter oxygen consumption, whereas insulin does.

Gastrectomy failed to cause pernicious anemia in five monkeys after an average time of 2.66 years (478 to 937 days) in the experiments of Bussabarger, Ivy, Wigodsky & Gunn (166).

Ball (167), using a capillary glass electrode, measured the pH

of various regions of the wall of the digestive tract of living albino rats. The wall of the duodenum was rather consistently acid, the ileum and cecum usually alkaline, and the colon approximately neutral. Flexner & Kniazuk (168, 169) described a method for continuous recording of gastric acidity.

Dennis (170) reports that contact of the mucosa of the ileum and jejunum with distilled water results in injury to the mucosa as shown by microscopic changes and changes in permeability and in failure of the intestine to do osmotic work.

According to Scatena (171), ptyalin is found in the saliva of the newborn in nearly all cases, even when premature. The amount present up to the fifth month is about one-quarter that found from the sixth to the twelfth month of life. Ingestion of food did not modify the concentration of ptyalin.

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LIVER AND BILE

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The functions of the liver are so numerous and varied and the fields of investigation relating to this gland are so extensive that it seems unwise to attempt to include in a brief chapter all the data that might come properly under the title "Liver and Bile." The writer, therefore, has limited the scope of this review to certain phases of liver and bile physiology. Fat and carbohydrate metabolism have not been included since several recent reviews cover these subjects in detail (1 to 8). Discussions of liver and bile physiology of interest to the clinician may also be mentioned (9, 10).

LIVER INJURY

Numerous experiments have been performed in an attempt to determine how the liver may be protected from injury by various toxic agents. Evidence has accumulated (11) that sodium xanthine accelerates healing of liver tissue in rats following carbon tetrachloride poisoning, in addition to preventing liver damage. In Fitzhugh's (12) opinion, xanthine injected subcutaneously protects the liver against carbon tetrachloride injury, as he found the extent of necrosis is limited to a small area about the central veins as contrasted to 50 to 80 per cent of the lobule in control rats. However, he could not demonstrate that xanthine exerts any influence on regeneration. According to Forbes (13) the development of liver cirrhosis from intermittent exposure of rats to carbon tetrachloride vapor over a period of weeks has been retarded in all cases and in a great majority of the animals cirrhosis was entirely prevented. In these experiments xanthine, sodium xanthine, or a crude liver preparation containing approximately 50 per cent xanthine, was injected subcutaneously.

Ravdin (14) and his associates have studied the effects on the liver of chloroform and note differences depending on whether the drug is volatilized by air or oxygen. Anoxemia during anesthesia results in a marked increase in the blood sugar levels above those obtained when anoxia does not exist. The prolonged hypergly-

cemia following the anesthesia is due to the effect of the drug *per se* on the liver cells. Liver glycogen concentration is steadily reduced during the period of narcosis since approximately 35 per cent is lost during the first two hours. At the end of a twenty-four hour period, 94 per cent of the liver glycogen has disappeared. The fatty acid concentration in the liver increases as the glycogen decreases. If oxygen is used to volatilize the chloroform, liver necrosis occurs to a lesser degree and the liver glycogen is more rapidly restored following anesthesia. Oxygen is believed to be as protective as carbohydrate against chloroform injury. These investigators (15), furthermore, report that the incidence and severity of damage to the liver by chloroform increase progressively with an increase in the concentration of lipids; this is independent of the content of liver glycogen at the beginning of the anesthesia. In their opinion, carbohydrates protect by reducing the lipid content of the liver and in cases of inanition the protection occurs by virtue of their protein-sparing action. A high protein diet on the other hand saves the liver from damage, even though its lipid content may be high. Their data indicate that the increased susceptibility of the starved rat to chloroform is mainly due to the animal's depleted protein stores. Ravdin and his associates (16) also confirm that xanthine is protective in rats. Evidence is also presented that sodium ricinoleate and colloidal carbon injected subcutaneously afford a high degree of protection to the liver. Sodium allantoin and caffeine decrease the incidence of hepatic necroses, but not the total number of injured livers. The common factor present in these experiments is the inflammatory reaction caused by the various above mentioned agents. It is possible that the liver is protected by the protein split products liberated from the body tissues because of increased protein katabolism incident upon the inflammatory reaction. This hypothesis is in accord with the known protective action of high protein diets. In another experiment (17), dogs were given a high fat diet for two weeks with resulting increase in the hepatic fatty acid concentration. The common and cystic bile ducts were then completely ligated and the animals were fed a diet high in protein and carbohydrate but without any fat. Such a diet was most effective in reducing the fatty acid content of the liver and in increasing the liver glycogen. These beneficial results were obtained in one-half the time that would be required for the reaction of the usual high carbohydrate diet. The

necessity of using a high protein-carbohydrate diet with limited fat is emphasized in the preoperative preparation of the biliary patient.

Experiments of Miller & Whipple (18) demonstrate that the liver injury due to chloroform anesthesia increases in extent as the protein stores of the body are depleted. In such a depleted dog fifteen to twenty minutes of anesthesia is frequently fatal, with accompanying extensive hyaline liver necrosis. This is in contrast to dogs having normal protein stores which may tolerate ninety minutes of chloroform anesthesia with but little evidence of injury. Such depleted dogs may be protected from injury by a single large meat feeding thirty-six hours prior to the anesthesia. Methionine (19), and to a lesser extent cystine, given by mouth or by vein shortly before chloroform anesthesia inhibits the liver injury in such protein depleted dogs. Since other nonsulfur-containing amino acids confer no protection, it is possible that the sulfhydryl groups play a role by combining with the chloroform and thus prevent the injury. A large meal of beef muscle or intravenous injection of plasma protein twenty-four to forty-eight hours before anesthesia confers protection and it is possible that the beneficial effects may be due to the sulfur-containing amino acids furnished by the material given to the protein depleted liver cells.

The importance of protein in protecting the liver against injury by drugs has also been confirmed by Messinger & Hawkins (20). Arsphenamine in doses of 0.03 gm. per kg. causes liver injury to a greater or lesser degree dependent upon the type of diet fed. Protein affords the greatest protection; carbohydrate is beneficial as well, but not as uniformly so. Fat proved to be deleterious, as the dogs developed progressive jaundice and severe liver injury, while some became intoxicated to the point of death. In certain instances dogs which had been protected by protein or carbohydrate developed jaundice when changed over to the fat diet, even when no additional arsphenamine was injected. Dogs severely intoxicated when changed from the fat diet to a protein or carbohydrate diet showed prompt improvement with clearing of the icterus. These authors agree with Ravdin and his associates that a diet rich in protein and carbohydrate, rather than just pure carbohydrate, is indicated in cases exhibiting liver injury. Bollman (21), however, found that rats exposed to carbon tetrachloride vapor for thirty minutes three times per week survived longer when given a high

carbohydrate diet. Fat was inferior to both carbohydrate and protein.

In Smith's (22, 23) opinion the toxicity of naturally occurring food selenium is largely determined by dietary factors. A level of intake of selenium, which is highly toxic and damaging to tissue when administered to rats in a diet of low protein and high carbohydrate, is only slightly harmful, if at all, when fed in a diet of high protein and low carbohydrate content. Injured rats show marked atrophic nodular cirrhosis, anemia, ascites, pleural and pericardial effusions with loss of hair. On the other hand, the same level of selenium given with a diet low in protein and high in fat caused only stunting of growth, and extensive loss of hair, but only slight fatty degeneration of the liver. Lewis, Schultz, & Gortner (24) have demonstrated that the toxicity of selenium (sodium selenite) added to diets containing 30 per cent casein is significantly less than when added to diets containing only 6 per cent casein. Cystine added as supplement to the low casein diet was of no benefit, whereas methionine caused improvement in growth and greatly delayed the lethal action of the element. Although these experiments do not deal with the effect of selenium on the liver, they add information indicating the importance of protein and its precursors in protecting the body against certain toxic agents.

Von Glahn, Flinn & Keim (25), investigating the effects of arsenates on the liver of rabbits, determined that hay and oats did not prevent injury. Carbohydrates, represented by white bread and potatoes, appeared to be beneficial since only a minimal amount of cirrhosis was found; hepatic necroses were rarely seen. Brewer's yeast (26) when added to the standard diet of hay and oats also reduced the amount of liver injury and life was greatly prolonged.

Of much interest is the report of György & Goldblatt (27) in which they describe the lesions developing in the liver of rats fed a basal diet deficient in the vitamin-B complex but supplemented with vitamin B₁ and riboflavin or with vitamin B₁, riboflavin, and vitamin B₆. The livers demonstrated parenchymatous and fatty degeneration, focal and massive necrosis, hyperemia and hemorrhage, and in some instances perilobular fibrosis. The addition of yeast or Peters' eluate to the diet regularly prevented this liver damage. Rich & Hamilton (28) observed the development of cirrhosis of the liver similar to the Laennec type in all of fourteen

rabbits which were kept on diets supplemented by various vitamins but lacking yeast. These investigators determined that the injury was due to lack of some factor contained in yeast other than vitamin B₁, B₂, B₆, or nicotinic acid. Both of these reports are highly significant since additional studies may establish the possibility that cirrhosis of the liver in certain instances is related to a deficiency state rather than to the effect of toxic agents.

Dill & Erickson (29) experimentally produced renal ischemia in pregnant dogs and rabbits and observed an eclamptic-like syndrome characterized by a rapidly fatal course with lesions in the liver and kidneys. Within 48 to 120 hours after ischemia was established, weakness, lassitude, coma, convulsions, hypertension, hematuria, albuminuria, and nitrogen retention developed. Death occurred in five dogs in from five to fifteen days and two others were killed after four and seven days. Two dogs that delivered twenty-four to forty-eight hours after operation recovered. The lesions in the liver ranged from cloudy swelling, dilatation of sinusoids, and hemorrhage to occasional periportal necroses and conspicuous focal necroses. The picture simulated that seen in eclamptic livers from human beings. It is conceivable that this abnormality may prove to be the result of disturbed protein metabolism.

Of interest is the report of McCulloch (30) that *Amsinckia intermedia* seed in the diet caused cirrhosis in pigs, horses, and a calf. If this seed would produce progressive cirrhosis in dogs, it would be of decided value as an experimental tool.

BILE

Studies relating to the formation of bile acids have been continued by Virtue & Doster-Virtue (31). These workers fed cholic acid to fasting dogs with bile fistulas for several days in order to deplete the livers of taurine by means of increasing the output of taurocholic acid. Cystine disulfoxide, cysteine sulfinic acid or cysteic acid was then given with the cholic acid, these procedures resulting in an increase in excretion of taurocholic acid. This suggests that these substances can be converted into taurine by the body. Similarly (32) cysteine and homocysteine caused the production of more taurocholic acid, but thioglycolic acid gave negative results.

The enterohepatic circulation of bile salts in the cat has been investigated by Mellanby & Suffolk (33). These studies disclose

that in the fasting cat 95 per cent of the total bile salt is contained in the gall bladder bile, while the hepatic bile, continuously secreted by the liver, contains only traces of bile salt. By contrast, when the cat is fed, the hepatic bile increases in its volume and bile salt content, but the gall bladder contains only a small amount of relatively dilute bile. If the small intestine is removed, there is an immediate fall in the cholic acid of the hepatic bile. The data indicated that the bile salts are more rapidly absorbed from the ileum than from the duodenum or jejunum and very little from the large bowel. When bile salts are injected intravenously, they are rapidly excreted in the hepatic bile and there is no evidence of storage.

Investigations of bile salt metabolism have been continued by numerous workers in Ivy's (34) laboratory. Utilizing bile-fistula dogs, they determined that with or without the return of bile to the duodenum, the bile volume and cholic acid output did not deviate more than ± 8 per cent from mean daily values. Cholesterol and bile pigment output, however, were subject to ± 20 per cent deviation. If the bile-fistula dog is fed three times daily without return of bile, the excretion of cholates, cholesterol, and bile pigments is quite constant. In contrast, the output of cholates is markedly increased when the bile is returned after each meal. When no bile is fed, 1.2 to 1.6 gm. of cholic acid are secreted daily; but when all the daily bile is fed to the dog, the level of secretion attains values between 6.5 to 9 gm. Although from 10 to 15 per cent of the cholic acid disappears during the enterohepatic circulation, the endogenously formed cholic acid compensates for this loss. In this way a more or less constant daily output is maintained. Bile or bile salt feeding increases the amount of cholesterol in the bile. These investigators (35, 36) have determined also the effects of various types of bile acids on the volume and constituents of the bile. Naturally occurring, unoxidized, conjugated bile salts when fed to a bile-fistula dog cause a moderate rise in bile volume output with increase in total cholates, nonvolatile solids, and viscosity. Ninety per cent of the cholic acid fed is recovered in the bile, usually within eight hours, and a proportionate increase in keto-reacting substances is also obtained. The increase in the latter substances may be the result of oxidation of the ten per cent cholic acid which is "lost" in the enterohepatic cycle. In contrast, when oxidized bile acids are fed, only from 9 to 37 per cent of the amount given can be recovered from the bile daily. There is, how-

ever, continued elimination of the oxidized bile acids from one to five days after cessation of bile acid feeding, so that 15 to 52 per cent may be eventually recovered. It is apparent that the body handles the natural bile acids in a different manner than it does the oxidized types. The oxidized unconjugated bile acids cause a marked increase in the volume and water of the bile with a decrease in content of cholates. Bile pigments are not affected by feeding bile acids, but bile cholesterol is increased by all of them except dehydrocholic acid which causes a decrease. These studies enable one to choose for administration that type of bile acid which will either cause a thin bile to flow or a bile which will be rich in the naturally occurring bile salts. Clinicians may find this desirable information in their treatment of patients. The lack of toxic effects on dogs or rats of long continued feeding of oxidized bile salts such as 'Decholin,' 'Ketochole,' or 'Dechacid' has been demonstrated (37). In another experiment these investigators (38) quantitatively studied the amount of keto-reacting substances in bile-fistula dog bile. Unoxidized bile acids when fed caused only slight increase, whereas the oxidized bile acids markedly elevated the amount of keto-reacting substances in the bile.

Josephson and his associates (39, 40, 41) confirm the fact that, if large amounts of sodium cholates are injected intravenously into healthy dogs, the major portion disappears from the blood stream within five minutes. The injected cholate can be quantitatively recovered in the bile within one to three hours, mostly in the first hour. The major portion regained from the bile in the first thirty minutes is mainly unconjugated, but in later samples of bile the concentration of conjugated acids rises considerably above the normal. Thus the conjugation of the bile acids in the liver is a time consuming process, possibly of enzymatic character. Similar results were obtained in human beings. These workers have also observed that elevation of bile acids occurs in the blood in obstructive jaundice in both rabbits and cats. The increase appeared later in cats because of the ability of the bile duct of cats to dilate. Augmentation of bile salts after cholate injection is much greater in these jaundiced animals than in normal ones and the elimination of cholates is much delayed. In phosphorus and carbon tetrachloride injury of the liver, the bile acid content of the blood is definitely increased, but not as high as in obstructive jaundice. Injection of sodium cholate results in augmentation in the blood and injury to

the liver cells interferes with their ability to secrete the bile acids and cholates.

Several clinical studies of human beings with disturbed liver function are of interest. Irvin, Johnston & Anderson (42) reaffirm the fact that concentration of all the bile acid fractions is very low immediately following removal of bile duct obstruction. A gradual increase to normal occurs throughout the recovery period. Desiccated hog bile given by mouth caused a marked rise in concentration of all the bile acid fractions but the increase in the desoxycholic acid group was greater than that of cholic acid. Unconjugated bile acids are present at all times but a decided rise occurs in the ratio of conjugated bile acids to total bile acids following the feeding of hog bile. This increase is mainly evident in bile acids conjugated with glycine. Gray, Butsch & McGowan (43) determined by means of T-tube drainage of the bile duct that the normal human liver concentrates 2,000 to 3,000 mg. of bile acids per 100 cc., whereas the damaged liver concentrates but 500 mg. In every case following operation, a postoperative inhibition of liver function occurred. This seemed to be unassociated with the duration of surgical procedure or with the type of anesthesia employed. The duration and degree of the postoperative febrile reaction paralleled the duration of the inhibition of hepatic function. These authors noted that increase in pressure in the bile duct caused a progressive decrease in the concentration of bile acids. In fifty cases studied by Gray and his associates (44) a low concentration of bile acids was found whenever there was other evidence of liver damage. There was postoperative inhibition in the function of the liver in concentrating bile acids but recovery of this function was prompt in all cases in which the liver was clinically normal. In cases with evidence of hepatic damage, recovery of concentrating ability of the liver was prolonged. It was observed that, whenever the amount of bile acid is constantly less than 200 mg. per 100 cc. of bile, the liver is severely damaged and the prognosis is grave. Several reports have been made by Zuckerman and his associates (45) on their study of a patient carrying a complete bile fistula. The daily bile output averaged 468 to 500 cc. or 10.5 to 11 cc. per kg. of body weight. This is the fasting rate when no bile was fed to the patient. The hourly rate of bile flow during day or night was fairly uniform, but during sleeping periods when metabolic processes are at a minimum appreciable diminution in the volume occurred. A high

protein diet consistently produced the greatest quantity of bile (46). This characteristic effect of protein has been reaffirmed by several other investigators (47, 48, 49). Intravenous injection of hypertonic 50 per cent glucose solution depressed the flow of bile (50). This is in accord with similar findings of Kocour & Ivy (51). When the patient was fed oleic acid added to an average mixed diet, the flow of bile was stimulated. Oleic acid plus bile salts caused an increase in volume greater than that resulting from feeding the two substances separately. In contrast, olive oil alone or with bile salts had no effect and glycerine increased the bile flow but slightly.

In the postoperative treatment of patients with biliary tract disturbance, Best, Hicken & Finlayson (53) utilize dehydrocholic acid to increase the pressure and flow of bile with consequent flushing of the bile ducts.

The gall bladder bile of various species shows a remarkable constant and characteristic hydrogen ion concentration which is not readily changed by therapy or food, according to Morrison and his co-workers (54). Ox bile in large doses, however, does change the reaction of the normally acid bile of dogs to alkaline and dilutes the constituents. Human cholesterol gall stones inserted into gall bladders of dogs decreased in size following the administration of large doses of extract of ox bile. This is thought to be the result of the chologogue effect rather than the change in hydrogen ion concentration of the bile. In human bile the hydrogen ion concentration is higher than normal when stones are present regardless of the type of stone.

Riegel, Calder & Ravdin (55) determined that cholesterol is not absorbed from hepatic bile placed in normal bile-free gall bladders of dogs when the ducts are completely ligated. However, if unligated accessory ducts to the gall bladder exist or if fluid is being secreted into the gall bladder lumen, the amount of cholesterol recovered is considerably in excess of that introduced. No relationship could be established between the concentration of cholesterol in the blood and the concentration of cholesterol in cholecystic bile. Johnson & Riegel (56) could not demonstrate any correlation between blood and bile cholesterol. When dogs were given large doses of desiccated thyroid by mouth or thyroxin by vein, no change in cholesterol concentration was evident in the bile, even though the blood cholesterol fell as low as 40 mg. per cent. High

levels of blood cholesterol followed thyroidectomy but concentration of the lipid in the bile was not altered.

Disturbances in fat absorption from the intestine of bile-fistula dogs was observed by Crandall & Ivy (57). No increase in blood fat occurred in a five-hour period after feeding 100 cc. of olive oil and water to a dog with bile fistula. Olive oil, however, when given with bile salts, caused an increase approximating the level found in a normal dog. Sodium dehydrocholate did not appear to be as effective in aiding absorption as the bile salts. Coffey, Mann & Bollman (58) determined the normal range of values for fat, nitrogen, and carbohydrate content of the feces of normal dogs. After ligation of the bile duct with exclusion of all bile from the intestine, definite diminution of the absorption of fat occurred. The amount absorbed depended upon the quantity of fat given. Nitrogen excretion in the feces was increased but carbohydrate utilization was not affected by absence of bile.

Haney, Roley & Cole (59) observed that whole bile or solutions of bile salts caused prompt and marked increase in the rate of propulsion of sponge rubber pellets placed in Thiry-Vella loops in dogs. Apparently, bile salts on the mucosa of the small intestine may play a role in the normal regulation of the propulsive movements of the small intestine. In biliary obstruction the lack of bile salts may cause interference with this mechanism and thus cause the delay in gastric emptying time.

Bachrach, Schmidt & Beazell (60) are of the opinion that the absence of bile in the upper intestine is responsible to a greater degree for duodenal ulcer development than is just loss of bile *per se*. In their bile-fistula dogs no ulcers were observed when bile was returned by mouth. In two dogs which developed ulcers, the bile had been returned through a tube into the small intestine.

The etiology of gall stones has been discussed in two critical clinical surveys (61, 62).

Bile pigment and hemoglobin relationships in newborn infants have been studied in detail by Waugh, Merchant & Maughan (63). The hyperbilirubinemia present at birth increases daily and then falls toward normal on or about the ninth day. Icterus develops in 30 per cent of the babies, appearing as a rule when the level of total bilirubin in the blood reaches five milligrams per cent or more. A continuous fall in hemoglobin and volume of packed red cells is also observed during this first nine-day period. No relationship

exists either between individual total fall in hemoglobin, volume of packed red cells, and the amount of bilirubinemia, or between the changes in hemoglobin, volume of packed red cells, and the total bilirubin on any particular day. Nor can any relationship be demonstrated between original height of hemoglobin level and the development of increased bile pigment in the blood. The hyperbilirubinemia and icterus neonatorum in particular are made possible by the increased blood destruction and whether or not icterus develops depends upon the functional ability of the liver cells to excrete the excess bile pigment. The blood gives both a direct and indirect Van den Bergh reaction. In this type of hyperbilirubinemia the indirect reacting bilirubin increases, whereas in erythroblastosis it is the direct reacting bilirubin that tends to rise. This latter finding suggests the development of an obstructive factor within the liver. Inspissated bile pigment is not uncommonly found in the bile canaliculi of the liver, indicating that the gland has been unable to cope with the great excess of bile pigment brought to it for elimination. In erythroblastosis the fragility of the red cells is increased and a severe anemia develops.

Laked hemoglobin injected intravenously into anemic dogs with bile fistulas is utilized to form new hemoglobin in an equivalent amount and concurrently there is a 90 to 100 per cent return of the injected hemoglobin in the form of bile pigment (64). It would appear that the pyrrole aggregates are split off from the hemoglobin with the formation of bilirubin, while the iron and globin fractions are utilized to form new hemoglobin. This evidence indicates that the body on demand can synthesize new pyrrole aggregates in considerable amounts. When dogs are constantly maintained in an anemic state, less bile pigment is formed, since more young red cells are kept in circulation and consequently fewer cells are disintegrating daily. It is suggested that one third to one fourth of the daily excreted bile pigment originates from muscle hemoglobin and the remainder from red cell hemoglobin.

Two new types of bile pigment compounds have been described by Lemberg & Lockwood (65). These substances are yellow in color originating respectively from biliverdin and mesobiliverdin by oxidation with two equivalents of iodine in the presence of zinc acetate. The names bilichrysin and mesobilichrysin are suggested. The absorption spectra of some bile pigments and of bile pigment compounds with iron have been studied by Holden & Lemberg (65a).

These complexes are intermediate products in the breakdown of hemoglobin to bile pigments. Spectra of these iron-containing complexes lack the Soret band found in closed ring compounds such as protohaematin and porphyrins, thus indicating that the compounds are of open ring structure.

Coolidge (66) maintains that the bilirubin in human plasma of high bilirubin content is bound to plasma albumin and that the character of the Van den Bergh reaction cannot be correlated with the concentration of cholic acid in the plasma. In his opinion the plasma bilirubin which gives a direct reaction is attached to the plasma albumin as a dissociable complex, whereas that portion which does not give a direct reaction is attached to a fraction of plasma albumin precipitable by ammonium sulphate—probably by a valence bond. Chakravarti (67), on the other hand, is convinced that the presence of bile salts in the plasma plays a role in determining whether the Van den Bergh reaction will be direct or indirect.

A new method for the study of urobilinogen in stools and urine has been described by Sparkman and normal values have been indicated (68, 69, 70). From the results of clinical studies he concludes that the estimation of urobilinogen in stools and urine is of value both in detecting and following the progress of hepatic disease and in the differential diagnosis of jaundice. Urobilinogen studies on stools afford an index of the rate of red cell destruction and aid in the differentiation between anemias of increased destruction and anemias of decreased production. Increases of urinary urobilinogen are consistently associated with the presence of bile pigments in the urine, an indication that pathologic urobilinogenuria does not occur as a result of excessive destruction of red cells unless there is concomitant hepatic damage.

A discussion of urobilins and urobilinogens has been submitted by Lemberg, Lockwood & Wyndham (71) and a method described whereby urobilin or urobilinogen may be distinguished from urobilin IX_a (mesobilinogen). In the opinion of these investigators the assumption that mesobilinogen is the mother substance of urobilin has no sound basis. Their studies disclose that urobilin forms at least 90 per cent of the total urobilins of normal urine and at least 80 per cent of the total urobilins in the majority of pathological urines. In abnormal urines, particularly of patients with

damaged livers, there may be urobilin IX_a admixed to urobilin and occasionally the former predominates.

PROTHROMBIN

Since the original reports by Warner, Brinkhous & Smith (72); Butt, Snell & Osterberg (73), and Dam & Glavind (74) which proved that hemorrhage in obstructive jaundice is due to prothrombin deficiency resulting from lack of vitamin K, innumerable confirmatory papers have been forthcoming (75 to 82). Experimental work (83, 84) had previously shown that lack of bile acids in the intestine of dogs was a factor in the development of prothrombin deficiency. Smith and his associates (85), after the discovery of the fat-soluble vitamin K by Dam, demonstrated that the prothrombin deficiency could be promptly corrected in bile-fistula dogs by feeding bile salt and vitamin-K concentrates. Greaves (86, 87) caused a similar deficiency to develop in rats either by ligating the bile ducts or by producing a bile fistula. Bile and vitamin-K administration cured this abnormal status; but if the rats were fed a vitamin-K-free diet, the addition of bile salts was not beneficial. If rats were given a vitamin-K-free diet for long periods of time and then the bile ducts were ligated, the hemorrhagic tendency developed promptly, indicating that the animals had no stores of the essential factor responsible for prothrombin production. Greaves also noted that only a few of his normal rats on a diet deficient in vitamin K would develop bleeding tendencies. Bile fistula, jaundiced, or normal rats raised on vitamin-K-free diets continued to excrete significant amounts of the vitamin, suggesting that bacterial synthesis of the vitamin was occurring within the intestine. Flynn & Warner (88) confirmed these results and in addition offered evidence that phthiocol and 2-methyl-1, 4-naphthoquinone were beneficial because of their vitamin-K activity. Thus the absolute necessity of bile salts in the intestine to aid in absorption of the fat-soluble vitamin K was firmly established.

Hypoprothrombinemia may develop if there is interference in the absorbing ability of the intestinal mucosa. Patients suffering from sprue, intestinal polyposis, or obstruction (89, 90, 91, 99) have been benefited by administration of vitamin K.

Several experiments substantiate the idea that the liver is the

site of production of prothrombin. Smith, Warner & Brinkhous (92) demonstrated that chloroform liver injury caused a reduction in prothrombin as well as in fibrinogen. Mild injury may affect prothrombin without altering the fibrinogen concentration. Warner (93) noted a decrease in prothrombin following partial hepatectomy with a return to normal values as the liver regenerated. A similar deficiency developed after complete hepatectomy (94, 95). Clinically, patients with biliary disturbance may not benefit from bile and vitamin-K administration because of persisting liver damage (77, 79, 96) or cirrhosis (97, 98, 99).

In brief, in order that vitamin K be effective in the treatment of hypoprothrombinemia there must be an intact liver, normal absorbing intestinal mucosa, and bile within the intestine.

Prothrombin deficiency in the newborn has been extensively studied. Smith and his associates (100, 101), using their two-stage method for prothrombin determination, revealed that newborn infants have very low prothrombin levels at birth with gradual increase toward the adult level, but it is practically a year before normal values are obtained. Lowest values are found from the second to sixth day of life. They state that in newborn infants the rapid convertibility of prothrombin to thrombin compensates for the deficient quantity of circulating prothrombin. Quick & Grossman (102) determined that prothrombin concentration is normal at birth but that a drop in values occurs at the end of the first day which persists until the sixth day when adult levels are again obtained. They suggest that recovery is brought about by establishment of bacterial flora in the intestine which initiates the synthesis of the necessary vitamin K. Differences in methods used explain the divergent data. The important fact has been established, however, that deficiency in prothrombin (103, 104, 105) exists in the newborn during the first few days of life. Hemorrhages are apt to occur during this period. In hemorrhagic disease of the newborn, prothrombin values are extremely low.

Waddell and his co-workers (106, 107) first demonstrated that vitamin-K concentrates are very effective in reducing the abnormally high prothrombin times in the newborn. Hellman, Shettles & Delfs (108, 109) have established that the feeding of vitamin K to expectant mothers results in an increase in the prothrombin of the baby. According to Salomonsen & Nygaard (110), the transi-

tory hypoprothrombinemia during the first five days of life can be corrected by the early feeding of infants from within two hours after delivery. Bacterial flora of the intestine thus become established with resulting synthesis of vitamin K. Prothrombin concentration (111) in the blood of the umbilical cord is low, although the values found in mother's blood (112, 113) are reported to be above normal.

The investigations of Tidrick, Joyce & Smith (114) reveal that the prothrombin of newly hatched chicks is low. Analyses made during summer months gave values at least 20 per cent higher than those performed during the late fall. This is an indication that the storage of vitamin K in the egg yolk varies with the season and depends upon the type of diet which was fed to the hens.

Dam, Glavind, Lewis & Tage-Hansen (115) have investigated the manner in which vitamin K is effective in maintaining prothrombin concentration. In their opinion, vitamin K is not a prosthetic group in the prothrombin molecule, but it must be present in the body tissues to promote production of prothrombin.

These are but a few of many references that might be cited in this extensive field of investigation. They illustrate the fundamental work that has been performed in the study of hypoprothrombinemia resulting from the various causes mentioned. Vitamin K has been reviewed in detail by Dam (116), and Grossman (117) has recently discussed the problem from the viewpoint of the pediatrician.

The story of the development of the synthetic compounds of the naphthoquinone group which have an effect similar to vitamin K when given by mouth or intramuscularly must be left for another chapter.

HEPARIN

The history, chemistry, physiology, and clinical applications of heparin have been discussed in detail by both Mason (118) and Murray (119). McClure & Lam (120) report on the nontoxic quality of purified heparin and discuss the fact that there is considerable variation in the amount of heparin necessary to elevate the clotting time to an arbitrary optimum level in different patients. Evidence is presented by Solandt & Best (121) that very extensive injury to arteries and veins never results in a maximal stimulus to platelet agglutination. The dose of heparin necessary to prevent

platelet thrombi in an injured vessel is much smaller than that required to prevent the process in a glass cell.

Studies of Jaques & Mustard (122) revealed that prolonged dialysis of plasma has little effect on the activity of heparin but that the addition of sodium chloride to this plasma decreases the activity of the heparin. These workers confirm the previous work of Howell which indicated that heparin is only active in the presence of a protein in plasma, which, although in the albumin fraction, is not crystalline serum albumin. Heparin is highly potent when plasma is used in testing its activity, but when a solution of fibrinogen is substituted, it is almost inactive. The addition of dialyzed plasma to the fibrinogen solution restores the activity of the heparin. In their opinion heparin acts by combining with and enhancing the action of normal plasma antithrombin.

Brinkhous and associates (123) state that the effect of heparin in blocking the conversion of prothrombin into thrombin requires the presence in plasma of an accessory inhibitory factor. Heparin alone does not block the formation of thrombin and the new factor alone has very little inhibitory effect. The two combined are highly effective. This plasma factor is nondialyzable and its identity has not been established as yet.

In a discussion of the antitryptic properties of heparin, Horwitt (124) reports that upon the addition of trypsin and heparin to plasma, these substances are mutually antagonistic. Clotting of the plasma will not occur unless the amount of trypsin added is more than enough to neutralize the effect of heparin. He suggests that since heparin has this antitryptic effect, it may now be possible to correlate certain increases in blood antitrypsin with increases in circulating heparin.

A good yield of crystalline heparin was recovered by Jaques & Waters (125) from blood obtained from dogs which had been sensitized to horse serum with subsequent induction of shock under anesthesia. This crystalline heparin appears to be identical with that which may be isolated from dog livers. They consider that this is conclusive evidence that heparin is responsible for the very prolonged coagulation time of blood in canine anaphylaxis. Jaques (126) also describes the preparation and properties of an enzyme derived from rabbit livers. This enzyme inactivates heparin and he therefore designated it as heperinase.

PLASMA PROTEINS

The role that the liver plays in the production of plasma protein has been elaborated upon by Madden & Whipple (127) and the reader may be referred to this recent review for details. Plasma fibrinogen response to various states in man has been discussed by Ham & Curtis (128). Consequently, the writer has limited the discussion in this field to the effects produced by acacia upon the plasma proteins. According to the reports of Knutti and his associates (129, 130, 131), acacia intravenously injected depresses the plasma proteins with accompanying marked reduction in the fibrinogen values. Repeated weekly injections of acacia maintain the low levels of plasma protein concentration and circulating plasma protein. After cessation of acacia administration, low values gradually return to normal after several months, and a portion of the acacia continues to circulate in the blood for long periods. The acacia accumulates particularly in the liver and the experimental data suggest that its presence in the liver cells interferes with plasma protein production. Acacia and carbon tetrachloride when given together cause greater depression of the plasma proteins than when either one is given alone. Bleeding may result from the severe reduction of fibrinogen. Although the plasma protein may be much below the edema level, no edema occurs because of the osmotic pressure exerted by the circulating acacia.

Similar results have been noted by other workers (132, 133). Falkenstein & Jackson (134) are opposed to acacia therapy because they observed marked decrease in the plasma proteins of a child under treatment for nephrosis. Low levels of plasma protein were maintained long after cessation of acacia administration. A total of 705 gms. of acacia was injected and marked enlargement of the liver occurred. Six years later upon examination of the viscera, 7.1 per cent of the total amount of acacia given was recovered from the liver. In their opinion the acacia within the liver cells may have interfered with plasma protein production.

Although the evidence suggests strongly that the presence of the acacia within the liver cells depresses plasma protein production, some other functions of the liver are not disturbed (135). The liver cells produce and secrete bile salts in a normal manner and there is no disturbance in the excretion of bile pigments. No alteration in the blood and bile cholesterol attributable to the acacia is

observed. The decrease in concentration of blood and bile cholesterol is the result of interference in fat absorption due to lack of bile salts and is normally found in long standing bile-fistula dogs.

However, the accumulating evidence indicates clearly that repeated intravenous injections of acacia are contraindicated as a therapeutic measure.

CLINICAL APPLICATIONS

Certain salient facts should be emphasized. As studies in protein metabolism continue, greater appreciation of the role they play in maintaining the body in a healthy state is steadily increasing. Evidence is conclusive that it is of the utmost importance that the body protein stores be kept replete in order that the tissues may be better protected from toxic agents elaborated within the body or coming from without.

Bile salt therapy is indicated for the patient suffering from biliary tract disease. By this means absorption of fat is facilitated and deficiency in the fat-soluble vitamins A, D, E, and K is prevented. The enterohepatic circulation of bile salts is also maintained and the resulting flow of bile continuously flushes the biliary tree with beneficial effects.

All patients with obstructive jaundice should receive vitamin K and bile salts or one of the water-soluble naphthoquinones with vitamin-K activity both before and after operation. Hemorrhagic tendency will thus be prevented since prothrombin will be maintained at adequate levels to assist in formation of firm blood clots.

Newborn babies should receive the benefit of vitamin-K administration. The vitamin may be given either to the mother shortly before delivery or to the child at birth. It is true that not all will necessarily suffer from lack of it, but the life of some infants with minor undetected hemorrhages may be saved. A minor hemorrhage, with consequent demand upon prothrombin, may exhaust the supply and major hemorrhages then occur, either resulting in death or in causing irreparable damage to essential organs. Methods suitable for laboratory or bedside determination of prothrombin are available to enable the clinician to evaluate blood prothrombin levels (136, 137, 138, 104).

Rapid strides in preventing thrombosis with consequent embolism are being made. The time is not far distant when heparin or some substance with similar activity will be universally available.

It will be a glorious day when the clinician can control thrombus formation and limit it to its salutary effects.

The dangers of chloroform as an anesthetic with its delayed liver damage cannot be emphasized too greatly. Its use should be reserved for emergency only. The same statement, in the opinion of the writer, applies to the utilization of acacia as a therapeutic procedure.

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FORMED ELEMENTS OF THE BLOOD

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This report is an attempt to review the literature appearing during the interval from July 1, 1939 to June 30, 1940, inclusive, on the formed elements of the blood. For comparison, a few papers appearing prior or briefly subsequent to that period are included. Emphasis has been placed on experimental studies, although occasional reference has been made to clinical data. Restrictions have necessarily been made and quite probably some papers have been overlooked; to authors of such papers I extend my apologies.

GENERAL

Use of experimental animals for study of blood dyscrasias has required exact data on the cytologic characteristics of the blood. The average number of erythrocytes in normal dogs (129) was recorded as 5.3 million per c.mm., hemoglobin, 12.6 gm. per 100 cc., and leukocytes, as 12,165 per c.mm. Age elevated the number of erythrocytes but depressed the number of leukocytes. Differences in breeds or contrasts between pets and stray dogs were not detected (129). In hogs, red counts of 5.46 million, hemoglobin levels of 13.55 gm., and leukocyte tabulations of 12,732 cells were recorded (127). Mean corpuscular volumes were 79.9 cu. μ , and the number of platelets was 194,000 per c.mm. Young Jersey calves have 8.7 million red cells and 10,674 white cells; after three to six years of age the red cell count dropped to 6.39 million, while the white remained the same (39). In cats, the red cell count was 7.24 million, the hemoglobin level 11.24 gm. (2); thus lower corpuscular hemoglobin values than in either dogs or hogs obtained. Kittens have fewer erythrocytes than the adult cat (69): 6.99 million, hemoglobin values of 7.7 gm., and leukocytes totaling 20,806. In normal rabbits (103) the erythrocyte range was from 5.01 to 7.36 million, hemoglobin from 67 to 88 per cent and total leukocytes from 7,100 to 14,700. In birds (163) the total number of red cells per c.mm. was 3.217 million; hemoglobin concentration was 12.68 gm.; and average leukocyte counts were 21,450. Thrombocytes were 23,400 per c.mm. of blood in pigeons.

Similarity exists between the form, size, and staining proper-

ties of the nucleated red cells of the marsupials, insectivores, bats, edentates, rodents, cetaceans, and ungulates (100); correlations were observed between the pulse rate, hemoglobin, and red cell numbers in lower vertebrates as well as in mammals (182). The number of red cells per c.mm. varied inversely as the size of the cell; corpuscular hemoglobin was correlated with the pulse rate (182).

For a comprehensive report on the origin and developmental potentialities of blood cells, the reader is referred to the lecture of Doan before the New York Academy of Medicine (43). Formation of blood cells in early human embryos is like that in other mammals (22). Mesenchyme cells contract into rounded, deeply basophilic cells, the hemocytoblasts. These give rise both intravascularly and extravascularly to primitive erythroblasts, macrophages and giant cells, and rarely to granulocytes. The megaloblast is of pathologic significance only (86). Its appearance indicates restriction in the proper activity of the antianemic principle in adult life, but it does not play a role in normal erythropoiesis. Megaloblastic blood formation (37) may result from (a) a deficiency in some specific gastric enzyme, (b) deficiency of extrinsic factor, (c) failure of absorption of antianemic factor, or (d) failure of bone marrow to utilize antianemic factor.

Definite patterns with regard to distribution of hemopoietic tissue and fat occur in the bone marrow of dogs (85). Three tendencies were discerned: (a) centripetal regression of red marrow, (b) rapid growth of metaphyses, and (c) formation of rapidly growing central fatty core. Titanium dioxide is rapidly phagocytosed in the marrow (85); but this process is restricted on unilateral ligation of the vascular supply. Recovery of such ischemic marrow occurred in four to five weeks. When the main blood vessel to the femurs of rabbits was interrupted (84), necrosis of marrow occurred adjacent to the course of the nutrient artery. Inflammatory exudates did not follow and repair was initiated by infiltration of new capillaries. Thorotrast is present in the bone marrow of dogs two years after the injection of 1 cc. per kg. (144). Larger amounts occurred in liver and spleen where pathologic changes indicated its toxicity. Rabbits were more susceptible than dogs (144). Rate of removal of thorium dioxide from the blood of rabbits is not always uniform (130). Testing samples of blood for thorium, by means of an ionization chamber, showed that in three to six hours after injection 75 to 85 per cent had been eliminated. Metabolism of slices of

rabbit bone marrow was not unlike that of normal tissues, especially lymphatic tissue. Respiration was about 70 per cent higher when slices were tested in normal serum than in Ringer phosphate glucose mediums (192). Metabolism of erythroid marrow was predominantly oxidative (193); that of myeloid was glycolytic.

Culture of bone marrow *in vitro* (136, 137) has physiologic and diagnostic value. Cultures indicate that granulocytes, lymphocytes, and monocytes follow distinct lines of development (87); intermediate forms between the three do not occur. Cultures provide quantitative data and give valuable information on histogenesis, duration of life, and lineage of cells. Data on effects of drugs, effective doses, and therapeutic possibilities of sulfanilamide and related compounds were obtained. Irradiation of marrow cultures indicated that promyelocytes are more sensitive than myelocytes and metamyelocytes (137).

ERYTHROCYTES

Biconcave shapes of erythrocytes disappear when blood is kept *in vitro* at body temperatures (50). If blood is kept flowing or shaken, such changes do not occur. This indicates definite interrelationships between cells and plasma at the interface. Surface tension of erythrocytes induced by needles held in a micromanipulator was recorded by a filar micrometer (134). Saponin, which induces hemolysis, decreased surface tension. The interior of the elliptic nucleated red cell of the newt is liquid and free of any detectable gel or supporting framework (134). By providing rest from the ionic changes in the circulation, the spleen prolongs the life of the red cell in cats and dogs (176). Yet electrophoretic potentials at the surface are no greater in erythrocytes from the spleen than in those of the splenic artery or femoral vein; changes were not observed in the electrophoresis of red cells sixteen months after removal of the spleen (176). Postsplenectomy anemia was regarded as an indication of the protective role of the spleen in safeguarding the integrity of the red cell. In rabbits, splenectomy increased the number of erythrocytes, the total leukocytes, and the sedimentation rate (181). Reticulocyte percentages were higher and the coagulation time was prolonged. Electrokinetic motility of erythrocytes is not modified by sensitization to hemolytic antisera or to cobra venom (5).

Increased fragility occurred in erythrocytes removed from the

spleen (187). This is not due to lack of oxygen or carbon dioxide, or to specific enzyme in the spleen, but rather to stasis in the circulation. Alterations in osmotic behavior of red cells occur when blood is kept *in vitro*, or in occluded spleen or kidney. Rat erythrocytes are normally more fragile than those of man (32); reasons for this are not understood. Corpuscular hemoglobin may have a bearing on fragility, for in rats fed an iron-free diet, hemoglobin values declined from 13.2 to 10.2 gm. per 100 cc., and the red blood cells were unusually resistant to hemolysis (32).

Present knowledge of the degree of permeability of mammalian erythrocytes to cations is controversial, but recent use of radioactive isotopes of sodium and potassium provides a new and more reliable approach to the problem. Heparinized blood samples removed from dogs after administration of radioactive sodium and measured for emission of gamma irradiation, showed that erythrocytes were permeable to the sodium ion and permeation resulted in a simple equilibrium (31). Permeability could not be correlated with lipid content of surface membranes. Using lipid-soluble substances, such as ammonium salts of fatty acids and carbonic acids, the amounts of lipid per red cell, per cc. of red cells, and per square micron of cell surface were determined (45). Lipid content of red cells was analyzed for cephalin, lecithin, cholesterol, and cerebroside values. No correlation was established between permeability and lipid content of the cell (45).

Atmospheric pressures and climatic effects modify the blood picture. Plasma volumes and cell volumes increased with rising temperatures but decreased with lowered temperatures (16). Plasma volumes changed more rapidly than cell volumes and hemoglobin levels, so that increases in blood volumes may be associated with temporary decreases in hemoglobin concentration and hematocrit values (16). Increased atmospheric pressures may accelerate rates of chick development but mortality is high (56). Allantoic blood vessels were underdeveloped, red cells were larger and corpuscular hemoglobin was less; no changes were observed in leukocytes. Increases in erythrocytes of 11 per cent and in hemoglobin concentration of 10.2 per cent occurred in dogs kept in a respiration chamber with oxygen content of 21 per cent and carbon dioxide from 2.5 to 5.0 per cent (123). Changes were not due to hemoconcentration, but to marrow stimulation induced by tissue anoxia. The effect of carbon monoxide on blood depends on the

manner in which it is given. If asphyxia was induced rapidly, no alteration in erythrocytes, platelets, or coagulation time was observed (146); if prolonged, then marked increases in red cells, platelets, and leukocytes occurred. These effects did not occur when the spleen had been removed.

Ether anesthesia induced an increase in erythrocytes, platelets, hemoglobin, and cell volume, but a decrease in coagulating time (165). Splenectomy reduced these changes by a half. Sodium iso-amylethylbarbiturate (sodium amytal) induced decreases in these values, not apparent when the spleen had been removed (165). Hyperventilation with mixtures of carbon dioxide and oxygen produced hemoglobinemia (179). If alkalosis was prevented by gastric lavage with 0.35 per cent hydrochloric acid, hemolysis was restricted, indicating a relation between pH and red cell destruction. Increased erythrocytes, hematocrit values, and serum protein levels occurring after periods of artificial fever, induced by an inductotherm (138), were due to splenic contraction. Temperature rises ranging from 2 to 4°F. induced contraction of the spleen, polycythemia, and plasma concentration; but these results were not observed when the spleen had been removed. Temperatures of 108° to 109°F. for longer periods induced marked changes. Lymphocytes became pyknotic, lymph follicles were replaced by granulocytes and clasmotocytes, and there was a progressive shift to the left in granulocytic nuclei of the peripheral blood (72).

In conformity with clinical observations on gun shot wounds, autogenous blood introduced into the peritoneal space, when soiling the peritoneum, gave considerable protection against peritonitis (92). Whole blood or hemolyzed blood, injected intravenously or intramuscularly, caused a slight increase in the number of red blood cells due neither to the hemoglobin nor to the bile pigment injected (160). Plasma-free erythrocytes from citrated blood increased hemoglobin values and raised the total erythrocyte count of rabbits (21).

Considerable significance has been attached to blood changes associated with adrenal insufficiency. Increases in erythrocytes, in hematocrit and in hemoglobin values have been correlated with lack of cortical hormone. Significant changes in these constituents of the blood of dogs following adrenalectomy were not detectable (194). Adrenalectomy in rats caused a rapid rise in erythrocytes and leukocytes appearing just prior to death (34). Erythrogenic

function has been assigned to a pituitary fraction, but data are inexact. A reticulocytogenic response followed injection of anterior extract into rats (10), but was not in proportion to amount given, while a diminution in red cell level was recorded. Support for the existence of a pituitary factor in hemopoiesis was afforded by a decline following hypophysectomy in both erythrocyte and hemoglobin levels (191). Fluctuations in leukocytic percentages prevented accurate appraisal of effects on white cells but bone marrow became progressively hypoplastic. Observations on experimental hepatic damage and its effect on red cells have been confirmed in the hog. Carbon tetrachloride by mouth induced pathologic changes in the liver, macrocytosis in the blood and, in some animals, anemia (126).

Inoculating sheep with culture filtrates of *Clostridium welchii*, types A, C, and D, produced divergent changes (62). Toxin of A produced a fall in red cells, hemoglobin and volume; toxin of D caused a rise. Type C was without specific effect on the blood. Type A caused an increase in sedimentation rate and fragility, resulting in anemia. Calves inoculated with the nodular worm, *Oesophagostomum radiatum*, became anemic; the red cells dropped to 2.5 million in five days (39). Eosinophilia usually accompanies *Trichina* infestation in man but in rats it did not occur (17) although there was an absolute and relative neutrophilia. Dogs infested with *Leishmania* show large quantities of organisms in histiocytes of the bone marrow (29). Lymph glands were enlarged; histiocytes and plasma cells occurred in lymph nodes as well as in the liver.

Experimental inoculation of rabbits with bee venoms reduced total red cells by two million, with inconstant hemoglobin values and inconstant increases in the leukocytes (40). When five to ten mouse units of cobra venom was given to rabbits five times a week for periods to eighteen weeks, changes in erythrocytes, total leukocytes, differential leukocyte counts, hemoglobin, blood sugar, or urea nitrogen were not observed (115).

RETICULOCYTES

Data on the time required for maturation of the red blood cell in the bone marrow and on the length of life of erythrocytes are conflicting. From cultures of bone marrow *in vitro*, slide preparations were made every two hours following explantation. Maturation time for reticulocytes was eleven hours, although it may

reach twenty-four (8); the average life span of the erythrocyte was considered to be sixty-five days. Removal of an amount of blood from a rat equal to 0.1 per cent of body weight induced a significant reticulocyte rise in twenty-four to forty-eight hours (73). Elevations proportionate to the amount of blood withdrawn were not always obtained, but conclusions were that the duration of life of the red cells in rats was eight to nine days.

Singer has advocated use of the rat reticulocyte reaction tests for bioassay of antianemic substance, but evidence concerning the suitability of the rat for such tests is conflicting. When stabilized white rats were used, the reticulocyte response to administration of human gastric juice, normal, heated, or neutralized, was not significant (162). Nicotinic acid, considered an essential food supplement for some animals, induced reticulocytosis when administered to young rats (54). Elevations in red cell counts did not occur; yet an increased percentage of reticulocytes, seventy-two to ninety-six hours after the vitamin had been given, was a genuine hemopoietic response. Reticulocytes obtained from rabbits, following hemorrhage, are no more resistant to osmotic hemolysis than normal red cells (20) in sodium chloride solutions of concentrations above 0.48 per cent; in lower concentrations the reticulocytes were much more resistant. It was not possible to obtain complete hemolysis of red cells, leaving reticulocytes intact.

HEMOGLOBIN

The capacity of the blood for oxygen absorption is a measure of the amount of hemoglobin. The oxygen content of blood may be determined by converting hemoglobin to acid hematin and measuring changes in oxygen content by the dropping mercury electrode (14). Recent observations indicate that a kind of hemoglobin exists in certain individuals (3) capable of binding carbon monoxide only after reduction with sodium hydrosulfite. Active oxygen-fixation power may not be reliable for hemoglobin determination. Hemoglobin varied with body weight (171). Increased values were found in man to accompany increased weight; in rabbits decreased hemoglobin values accompanied weight increases. Not all liver substance is equally valuable for hemoglobin regeneration. Livers of lower vertebrates fed to standardized anemic dogs were of less value than those of mammals (157). Three hundred grams of shark liver, fed daily for two weeks, produced 57 gm. of

hemoglobin; the same amount of hog liver produced 91 gm. Reptilian liver contains liberal amounts of hemoglobin-producing factor, amphibian liver less, and piscine liver least of all. Protein intake modified hemoglobin regeneration in dogs made anemic by bleeding (64). If protein intake was low, so that production of globin was restricted, hemoglobin levels remained low. An abundance of iron will not compensate for low protein intake. Seven or eight grams of protein in the diet will regenerate one gram of new hemoglobin (64).

The relation of porphyrins to hemoglobin is a matter of controversy. Porphyrin metabolism in lead poisoning (190), pernicious anemia, and hemolytic jaundice is known, but little has been learned of the relations to leukemia and various anemias. Coproporphyrin I and erythropoiesis in bone marrow are interdependent, while coproporphyrin III denotes a disturbance in the formation of hemoglobin (194a). Chlorophyll is a source of porphyrin for hemoglobin in herbivora (95), but may not be used by carnivora. On a synthetic diet low in porphyrin erythrocyte values may drop from 5.2 to 3.6 million and hemoglobin from 10.5 to 7.1 gm. Water-soluble chlorophyll restored normal blood levels. Cats, when placed on a diet low in porphyrin, did not become anemic unless bled. When anemic, addition of chlorophyll to the diet did not raise blood levels (95).

Bilirubin will accelerate blood regeneration. Rabbits, made anemic by bleeding, recovered normal blood levels more rapidly when one milligram of bilirubin was given intravenously per day (207). Amounts of bilirubin from 25 to 30 mg. induced a sustained elevation in the number of red cells of patients with hypochromic anemia (208). In anemic dogs with biliary fistulas, the output of bile pigment and regeneration of hemoglobin were determined after injecting laked hemoglobin (76). Bile pigment was formed from the pigment radical or pyrrol aggregate, split off from the injected hemoglobin, in the ratio of 35 mg. of bile to 1 gm. of hemoglobin; the iron and the globin fractions were retained and used to form new hemoglobin, with new pyrrol aggregates formed by the body (76).

Amino acids, in doses of one gram daily, stimulated regeneration of hemoglobin in standard anemic dogs (201, 156). Glycine, glutamic acid, aspartic acid, cystine, histidine, phenylalanine, and proline increased hemoglobin production by 23 to 25 gm.; alanine and valine increased it but 13 to 17 gm. The natural *D*-form of

valine stimulated hemoglobin; but its optical isomer, the *l*-form, was less potent (158). Isovaleric acid was more effective than *l*-valine (158). Sodium chloride, 5 to 15 gm. in 500 cc. of water, decreased hemoglobin concentrations 11.2 per cent (166), owing to a temporary increase in volume by fluid passing from the tissues into the blood. Gastric mucin stimulated hemoglobin regeneration in anemic dogs 55 to 70 per cent more than the amount regenerated by iron ammonium citrate (202), in which the iron content was equal to that of the mucin.

The need for copper with iron for maximal hemopoiesis has been confirmed. Anemia was induced in dogs, maintained since birth on a milk diet, by bleeding (57). Liver restored normal blood levels in four weeks. In such dogs fed iron and copper, blood regeneration was rapid, and hemoglobin levels, approximately equal to those following liver feeding, were observed. Iron without copper induced hemoglobin levels a third as high as those seen when copper was given with the iron. When copper stores were reduced to low levels, iron utilization was practically absent. Comparable results occurred in rats (104). Copper-fed anemic rats, maintained on a milk diet, produced a small increase in hemoglobin. Iron produced only slight elevations, but copper with iron induced marked regeneration and improvement in growth and reproduction (104). Copper acts as a catalyst, securing the effective action of iron. Copper concentration in the blood was not elevated after severe hemorrhage in sheep (42), although it had previously been shown to be elevated in rabbits, dogs, and pigeons.

Free iron in the blood may be derived from the reticulo-endothelial system. An injection of cell-blocking substance not containing iron lowered levels of free iron in the blood (190); but repeated injections, resulting in a stimulation of the reticular apparatus, elevated free iron. Such increases may well be associated with hemopoietic stimulation. When red cells were incubated in their own plasma at 37°C., a migration of iron and bilirubin from cells into plasma occurred (12). The amounts of iron were in excess of the amounts of bilirubin. Red cells may be the source of the iron in plasma (12), and the "easily split off" iron may be intermediate in bilirubin production. There is probably no specific enzyme in the plasma effecting the conversion of hemoglobin to bilirubin. Exact data on the metabolism of iron are becoming increasingly available owing essentially to the use of radioactive isotopes (65, 124). Radioactive iron, in the form of citrate, chloride, or sulfate, was fed

daily to standard anemic dogs. Radioactive iron appeared in the red cell within four hours and within four to seven days it was entirely converted into hemoglobin (67). The percentage of iron absorbed is greater when smaller amounts are provided. Of amounts ranging from 1.2 to 4.9 mg., 47 to 60 per cent may be absorbed (67). When there is need for iron by the body, larger amounts are absorbed; but the mechanism regulating the absorption of iron is not known. Small amounts of radioactive iron escape from the body through the urine and through the feces; small amounts occur in the bile (66). The body controls its iron stores by absorption rather than by elimination (66).

Abnormal pigments appear in the blood. A review is available which includes a description of spectrophotographic methods for the determination of methemoglobin and sulfmethemoglobin (122). After sulfanilamide therapy methemoglobin occurred more frequently than sulfmethemoglobin (75). Methemoglobinemia may follow a single dose or occur hours after the blood sulfanilamide concentration has reached a peak (75). Sulfhemoglobinemia may follow long courses of sulfanilamide therapy but bears no relation to the concentration in the blood. Some new substance, functioning as an oxidant, may cause the production of both methemoglobinemia and sulfhemoglobinemia. The formation of these pigments in tissue slices of liver, kidney, muscle, and spleen, suspended in Ringer phosphate with various concentrations of sulfanilamide, was studied (74). Sulfanilamide does not act directly on hemoglobin, but by tissue interaction some oxidant induces the formation of the pigments (74). Cyanosis developed in animals given large amounts of sulfanilamide (114); less occurred after neoprontosil, and none after giving sulfapyridine. Following sulfanilamide there was an increase in methemoglobin, a greater increase in sulfmethemoglobin, and a decrease of 10 per cent in total hemoglobin (169). Slight changes followed sulfapyridine. Methemalbumin is a known extracorporeal pigment. It occurs in the plasma in certain types of anemia, or on incompatible transfusions (51). It is formed from extracorporeal hemoglobin; the heme fraction unites with serum albumin to form methemalbumin (51).

THE EFFECT OF DRUGS

Administration of sympathicolytic drugs (yohimbine, ergotamine, diethylamino-ethyl-ether, and so forth) had no significant

effect on erythrocytes (11); but, with the exception of ergotamine, all produced a fall in leukocytes, followed by a marked neutrophilia. Epinephrine and ephedrine, sympathomimetic drugs, caused contraction of the exteriorized spleen (142). Amphetamine sulfate (benzedrine sulfate) in doses of 1 mg. per kg. had similar effects (142). The blood pressure rose and the spleen reached its maximal contraction in thirty minutes. Erythrocytes rose 2 to 29 per cent but this increase did not occur when the spleen had been removed. Amphetamine sulfate in doses from 20 to 200 mg. per kg., given subcutaneously or by mouth to guinea pigs, rabbits, monkeys, dogs, and sheep, induced varying degrees of anemia accompanied by granulocytosis (47). Anemia, varying with the dose, was most marked fifteen to twenty-two days after administration of the drug. Saponin in amounts of 0.50 to 0.75 mg. per kg. produced hemolysis (81). Recovery ensued but too rapidly to permit assay of liver preparations. Collargol stimulated recovery following saponin hemolysis. Human serum contains some substance which inhibited hemolysis induced by digitonin (52); but its effectiveness depends entirely on the well-being of the individual whose serum was tested.

Sodium iodoacetate did not produce in dogs conditions similar to adrenal insufficiency, but a rapid and fatal anemia with increased functional activity of the bone marrow occurred (199). Choline hydrochloride depressed an active hemopoiesis and is as effective as raw liver (38). Experimental polycythemia was induced in dogs by the treadmill, by cobaltous chloride, or by confining animals in low pressure chambers with pressures reduced to 430 mm. of mercury. Erythrocyte levels increased 20 to 30 per cent. Eight milligrams of choline hydrochloride daily restored blood levels even in continued presence of polycythemic stimulation (38). Seventy-five grams of whole raw liver is as effective in depressing hemopoiesis as choline. Splenic changes were followed in chronically morphinized rats during the stages of the morphine cycle (111). Macrophages increased in number, size, and activity during the active phase, but decreased during the craving phase. Lymphocytes and erythrocytes arose from undifferentiated reticular cells, and erythrocytes arose within endothelial sinuses without passing through extravascular development (111).

Cobalt in the nitrate or chloride form (38) stimulated erythropoiesis, resulting in experimental polycythemia in normal animals or those made anemic by repeated bleeding or by benzol (98).

Daily administration of cobalt induced high erythrocyte levels and hyperplasia of bone marrow with increase in megakaryocyte-like cells which appeared to be centers for erythrogenic proliferation (98). An intense hyperleukocytosis with elevations in monocytic percentages followed small doses of metallic cobalt (131). Similar results occurred when cobalt chloride was given to young rats rendered anemic by an exclusive milk diet (147, 148). Hemoglobin values increased and the number of erythrocytes rose from 4.68 to 7.30 million. Cobalt chloride given to dogs made anemic by nutritional restrictions induced severe anorexia, diarrhea, and weight loss (148). Some gain in red cells and hemoglobin values was observed, but less than was observed in mice following cobalt. Copper and iron were more effective than cobalt.

Intravascular destruction of leukocytes followed benzol administration (79). Hypersegmented, degenerative leukocytes were seen in capillaries of bone marrow, adrenal, and hypophysis; phagocytosis was most abundant in liver and lymph nodes. The effect of aminopyrine was not unlike that of benzol (27). Destruction of myeloid elements preceded aplasia of erythroid cells, but the drug appeared to have a selective toxic action on bone marrow.

Small doses of heparin did not induce anemia; but larger doses caused a 50 per cent decline in erythrocyte and hemoglobin levels (77). Organic changes did not appear. Heparin is inhibitory to growth of undifferentiated cells; prothrombin is growth-promoting. Heparin and prothrombin appear in abundance in the body, and it is postulated that they may regulate processes other than coagulation (77). Leukopenia was induced by eighty grams of indole when given to rabbits in 4 cc. of 40 per cent alcohol (183). Indole depressed leukopoietic marrow, but a marked reticulocytosis accompanied the anemia. Fluorides, oxalates, and arsenates produced progressive swelling of the red blood cells with consequent hemolysis, but effects of oxalates and arsenates occurred with less regularity than those of fluorides (68). Nucleated red cells of the turtle and the snake were not susceptible to fluorides. Studies of cellular percentages in bone marrow were made after (a) repeated bleeding, (b) lead acetate, (c) collargol-saponin, and (d) in animals on deficient diets (35). Emphasis on bone marrow changes, accompanying experimental anemia, was stressed. Epinephrine in doses of 0.7 mg. stimulated leukopoiesis in man (18). If an impetus toward myeloid proliferation was present as in leukemia, these trends were greatly accelerated by epinephrine.

Chemotactic response of leukocytes for pneumococci was altered by alcohol (99). Intoxication by ethyl alcohol, in degrees ranging from motor instability to deep coma, was induced and chemotactic indexes set up on blood smears showed that polymorphonuclear leukocytes of deeply intoxicated rats lose their positive chemotaxis toward pneumococci.

Reports have appeared of blood changes after therapeutic doses of sulfanilamide and sulfapyridine. No attempt will be made to review them. Experimentally, low grade anemia was induced in dogs by giving 0.2 gm. per kg., over a period of three months (139). Concentrations in the blood of sulfanilamide, sulfapyridine, and diaminodiphenylsulfone sufficient to induce definite effects on the erythrocytes in mice (153), were, in the order named, 2.7 to 4.9, 7.4 to 10.6, and 1.5 to 2.4 mg. per 100 cc. Sulfapyridine is half as effective in producing red cell destruction as sulfanilamide; diaminodiphenylsulfone twice as effective. Blood changes were produced by giving sulfanilamide by mouth (113, 114). Progressive anemia with cyanosis was induced, the intensity depending on the amount given and the duration of treatment. There was reticulocytosis, macrocytosis, anisocytosis, and leukocytosis. Hepatomegaly and splenomegaly were common. Bone marrow reactions indicate an initial myeloid stimulation, followed in six days by a greater erythroid stimulation (80). The myeloid-erythroid ratios, normally greater than 1.00, were less than 1.00 in animals receiving sulfanilamide. Eosinophilic stimulation in the bone marrow was not observed. Sulfanilamide stimulated phagocytosis of bacteria *in vitro* and the rate of migration of rabbit leukocytes; likewise, in cultures of buffy coat, the migration of leukocytes was 10 per cent greater when neoprontosil, in concentrations of 1:1000, was added to the explants (78).

ANTIANEMIC FACTORS

The cause of pernicious anemia is as yet unknown. The disease has not been produced experimentally; the causative factors are thus not clearly defined, but some interruption in the sequence of normal processes which elaborate, store, and utilize antianemic factor occurs. Six stages have been identified: (a) extrinsic factor, supplied by food; (b) intrinsic factor, produced by pyloric gland organ; (c) interaction between the two; (d) absorption; (e) transport to liver and other organs, and (f) storage (120). Localization of antianemic substance in the gastrointestinal tract varies as be-

tween men and animals. In man, the pyloric portion of the stomach has been combined with the Brunner's gland area of the duodenum to form the pyloric gland organ (121). Intrinsic factor is presumably elaborated here. The small intestine and colon of swine will stimulate blood regeneration (41). Repeated washings removed the effective factor; accordingly it was concluded that it was only adsorbed and not elaborated in the intestine. Loss of the gastric principle by total gastrectomy in monkeys did not produce pernicious anemia after 2.6 years (26). Mild oligocythemia, with hypochromic normocytic anemia, developed. Iron in citrate form every other day for one month restored normal blood levels. Removal of seven-eighths of the stomach, all of the duodenum, and 30 cm. of the jejunum from dogs did not result in anemia of the pernicious type in two years (9). The so-called pyloric gland organ is not essential for the maintenance of normal blood pictures in dogs.

Data assembled from studies of fetal and new-born blood, after administration of antianemic principle to the mother, are inconclusive. Liver extract given parenterally to pregnant rats, in large amounts, failed to induce significant changes in the blood of the young, at birth or at frequent intervals thereafter (24). Normal human gastric juice, given to pregnant rats, accelerated hemopoiesis in the fetus, whereas that of patients with pernicious anemia had no effect (161). Gastric juice of patients with simple achlorhydria produced variable results; that of patients with carcinoma of the stomach provided some hemopoietic stimulation (161). Anti-anemic principle may pass the placental barrier. Extracts of fetal bovine livers produced prompt and definite remissions in patients (203). Ventriculin, fed in appropriate amounts, produced definite changes in size and nuclear characteristics of eleven-day rat erythroblasts (81). Concentrations of 20 per cent were ineffective; small percentages produced significant changes. Large amounts may have produced toxic conditions interfering with absorption. Immature opossums, nourished in the marsupial pouch of the adult, were injected with normal human gastric juice and concentrated liver extract (172). Significant reductions were observed in corpuscular volumes and in maximal diameters of developing erythrocytes. Argentaffin cells may elaborate the principle effective against pernicious anemia (88). These cells occur in the gastrointestinal tract of all mammals and are abundant in the pylorus and duodenum, the pyloric gland organ. They are absent, or prac-

tically so, from the gastrointestinal tract of the patients with pernicious anemia.

NEUTROPHILIC LEUKOCYTES

A rapidly fatal epizootic disease, inducing vomiting and progressive anorexia, has been observed in cats (70, 71). Hematologic changes include (a) decrease in lymphocytes, (b) degeneration of germinal centers, and (c) intranuclear inclusion bodies in follicles. Immature myeloid and erythroid cells in bone marrow contain eosinophilic intranuclear inclusion bodies. Decreases in peripheral neutrophilic leukocytes follow myeloid pathologic changes in the marrow, although changes in the erythrocyte count are insignificant. Vaccination by injection of suspensions of infected tissue induced resistance and prevented appearance of the disease (48). When survival occurred, there was marked myelogenous leukemoid response. Feline agranulocytosis has many points of similarity to human agranulocytosis (107). The marked "shift to the left," absence of thrombopenia and extensive anemia, and sudden drop in neutrophilic leukocytes are similar in the two. Leukopenia is the result of (a) arrest of myeloid differentiation, and (b) destruction of granulocytes in circulation (71).

Healing operates independently of polymorphonuclear leukocytes. Neutropenia was induced by the intraperitoneal injection of antileukocytic sera (106). Tensile strength of repair of an abdominal wound indicated that fibroplasia proceeded independently of leukocytes and repair occurred equally well in neutropenic as in normal animals. Leukocytosis which accompanied inflammatory reactions was not induced by leukotoxin, the nitrogenous substance liberated in regions of inflammation (118). There is a leukocytosis-promoting factor in inflammatory exudates, which acts on marrow, inducing the formation of immature myeloid cells. It is neither histamine nor nucleic acid (117). It is thermolabile; heating to 60°C. inactivates it. Pleural exudative material from a dog, injected by cardiac puncture into a normal dog, induced leukocytic elevations of 77.2 per cent (118). Fractionation, after dialysis or by means of ammonium sulfate, indicated that it is a globulin primarily linked with the pseudoglobulin fraction derived from protein catabolism (118). Neutrophilic leukocytosis was produced by sodium nucleinate or pentose nucleotide (132). Marrow biopsies showed a shift to the left, and myeloid-erythroid ratios changed from 75:25 to 95:5.

In experimental lobar pneumonia in dogs, the alveolar exudate changed from a polymorphonuclear to a mononuclear one. Mononuclear cells displayed a greater phagocytic activity toward pneumococci and their ability to digest organisms was much more marked (155). Destruction of the organism by polymorphonuclear cells hinged on optimal concentrations of the opsonic properties of the body fluids, whereas mononuclears destroyed organisms in opsonic concentrations in which polymorphonuclears were relatively inert (155). Glycogen content of the red blood cell was relatively constant, but variations were found in glycogen values of leukocytes (25). Glycogen values increased after the injection of epinephrine or of glycogen.

Granulations of polymorphonuclear leukocytes may be of diagnostic value. Sputa of tuberculous patients induced changes in granulocytes of guinea pigs (19). Changes in color of granulations, in appearance of cytoplasm, and in size and volumes of cells indicated diagnostic possibilities. Injection of a sterilized suspension of ventriculin into rabbits and guinea pigs produced a marked increase in the basophilic leukocytes (143); basophilic myelocytes occurred in the loose connective tissue. Basophilic leukocytes were presumably derived from bone marrow; basophilic myelocytes from polyblasts. Toxicity indexes of various germicides were established by testing phagocytic activity of cells, mixed with concentrations of a germicide, toward opsonized staphylococci (198). The lowest concentration of a germicide which caused complete inhibition of phagocytosis was the "toxic endpoint" for that antiseptic.

LYMPHOCYTES

Evidence against the unitarian concept is recorded in the differentiation in manner of locomotion between lymphoblast and myeloblast. Locomotion patterns of stem cells from cultures of marrow, lymph node and buffy coat of lymphoid and myeloid leukemia were not alike (151). Movement in tissue culture served also to differentiate cells of the spleen reacting to foreign proteins (152). The splenic tumor cell is not a histiocyte, is not phagocytic, and its motion is not that of the myeloblast. It is precisely that of the lymphoblast. Lymphocytes, reacting to foreign proteins, play some role in antibody formation (152).

The fate of the lymphocyte is entirely unexplained. More small lymphocytes are lost daily than are present in the blood stream at

any one time. Autogenetic or heterogenetic lymphocytes when injected into the blood stream of rabbits from which the gastrointestinal tract has been removed, disappeared as rapidly from the circulation as in controls on which operation had not been performed (49). Their elimination was effected in ways other than by the gastrointestinal tract. Aggregation of lymphocytes occurs in the subosteal areas of the bones of chickens, pigeons, and turkeys (90). Lymphocytes filter from the blood stream into the subendosteal sinusoidal rete and thence enter the so-called erythrocytic capillaries. Lymphocytes, thought lost, may be potential erythroblasts. Small lymphocytes may become thrombocytes; larger ones, erythrocytes, leukocytes, or monocytes (90). Lymphocytes in peripheral lymph ranged from 280 to 370 per c.mm. (206). Thoracic duct lymph contains approximately 9,040 cells per c.mm. It is estimated that of every thirty lymphocytes which enter the blood stream twenty-nine are newly formed in lymphoid tissue. Thoracic duct lymph is sufficiently rich in cells to replace all lymphocytes in the blood stream twice daily; many are destroyed or lost or may transform into stem cells of myeloid and erythroid series (206). Evidence against the filtering of lymphocytes into marrow for erythrocytic purposes is submitted in a qualitative study of the hemopoietic organs (94). Quantitative statistical appraisals, coupled with study of mitotic cycles, suggest that both spleen and bone marrow supply sufficient cells for their own needs, and that filtration into bone marrow of lymphocytes for erythrocytic or myelogenic purposes (90) does not occur in mammals (94).

Subdermal areas in rabbits were chronically and acutely inflamed by the injection of egg albumin (102). Alterations in the nuclear and cytoplasmic structure of the lymphocytes involved transformation of the pachychromatic lymphocytic nucleus into a leptochromatic macrophagic nucleus, presumably through edema. Lymphocytes play a marked role in the resistance of the body to the action of tubercle bacilli, by disposing of the bacterial toxins (188, 189). Their defense is not due to extracellular liberation of agglutinins, metabolites, enzymes, or lysins. When sputa of pneumonic patients were added to suspensions of lymphocytes and injected into experimental animals the reaction of the lymphocyte against pneumococci was clearly established. Lymphocytes function in resistance to pneumococcal types III and IV by ingestion and intracellular disposition.

The origin of megakaryocytes has been ascribed to lymphoid cells, hemocytoblasts, hemoblasts, reticular stellate cells, myeloblasts, and so forth. In adult mice a large lymphoid cell, with basophilic cytoplasm and large vesicular nucleus in myeloid and lymphoid tissue, was regarded as the precursor of the megakaryocyte (149). This hemocytoblast differentiates into the adult form by chromosomal multiplication without cellular division, to produce the giant polyploid cell with polymorphous nucleus. Megakaryocytes probably do not arise in the lung (91), as recently supposed; lungs are probably not the chief source of platelets. Sections of lung and of related blood vessels and smears of blood specimens indicate that the lung serves only as a filter to remove from the circulation degenerate megakaryocytes and giant nuclei extruded into the circulation from the marrow (91).

MONONUCLEAR LEUKOCYTES

By visual and photographic observations on preparations grown in Sandison-Clark transparent windows, monocytes were observed to leave the blood vessels, migrate to a clot, phagocytose debris, increase in size from ten to thirty microns and assume all appearances of tissue histiocytes (46). Conclusions are indicated that blood monocytes develop into cells indistinguishable from histiocytes. After injecting India ink intrasternally, monocytes bearing ink granules were seen in the circulating blood; it was concluded that these cells were derived from the phagocytic reticulum marrow (133). In monocytosis, transition forms between reticular cells and typical blood monocytes may be seen in the circulation.

Pneumococci in the blood stream increase the percentage of mononuclear leukocytes. The injection of 0.25 cc. of Type I into guinea pigs caused a decrease and then a marked elevation in the percentage of mononuclear leukocytes (55). This elevation does not in itself indicate recovery; guinea pigs may die with high mononuclear levels and there seems no clear interpretation of the relation between mononuclear response and recovery. Mononuclear reaction to the organism indicates probably a definite specific cytologic response to certain lipids (186). The phosphatide fraction elicited infiltration of mononuclear cells; the purified cerebroside was slowly absorbed and elicited a reaction largely polymorphonuclear. A mixture of the two induced a response largely mononuclear but differing considerably from that which followed

phosphatide fraction. Cowpox lymph with human serum elevated the monocyte percentage 26 to 40 per cent (128). These cells were pathologic, both nuclei and cytoplasm being frequently vacuolated and azure granules being less abundant. Mononuclear cells from pleural exudates, removed seventy-two hours after aleuronat-starch mixtures had been injected into the chest, provided definite resistance to intrapleural infection against the *Pasteurella* group of organisms (196). Exudate cells, almost exclusively polymorphonuclear, derived about eighteen hours after an aleuronat-starch injection, had but slight effect against a fatal dose of *Pasteurella lepi-septica*.

Observations of growth in transparent chambers inserted into rabbit ears show how rapidly connective tissue regeneration may close a wound (174, 175). In ten to thirteen days an entire fibrous sheet may cover an area 6.0 mm. in diameter. Fibroblasts first appear and then unite by broad bands to form a syncytium. Fibrils are laid down extracellularly by fibroblasts. Reticula of fibrils develop around fibroblasts and in forty-eight hours may almost obscure all fibroblasts. Interaction of fibroblasts and sarcoma cells with leukocytes and macrophages *in vitro* suggests that leukocytes produce materials which stimulate growth of fibroblasts (109). Growth begins sooner and continues longer, and symbiosis between the two cells seems shown. Sarcoma cells do not attain the same proportions of growth as fibroblasts, owing possibly to accumulation of toxic metabolites in cultures. It is postulated that leukocytes, essentially monocytes and macrophages, stimulate growth of malignant cells and facilitate their invasive tendency (109).

EXPERIMENTAL LEUKEMIA

The cytology, general morphology, and transmission of leukemia in a highly inbred pedigreed strain of mice, the F strain, is reported (96). Leukemia developed from introduced cells and not from local reticulo-endothelial or mesenchyme cells. Cells showed wide degrees of cytologic variation, with no specific cytologic criteria for malignancy. The concept of leukemia as a neoplasm or as a hyperplastic response of multicentric foci is still unestablished. Inoculating mice with different neoplastic lymphocytes resulted in cellular gradations between lymphosarcoma and leukemia (97). Cytologic differentiation between the myeloid types of leukemia and the nonmalignant extramedullary myelopoieses was demon-

strated (13). Myeloid cells are immature in leukemias; all forms may appear in myelopoieses. Differences were observed with respect to (a) preponderance of erythrogenic foci, (b) percentages of megakaryocytes, (c) size of the liver, (d) transmissibility, and (e) bacterial relationships (13).

Susceptibility to transmissible leukemia is a dominant (58), but data do not permit a precise statement of the mode of inheritance. Differences occur between the manner of inheritance of spontaneous and transmitted leukemias, involving perhaps multiple Mendelian factors (58). Semidominant genes or simple recessive genes, together with some localization factor, may be responsible (164). Inoculating two strains of mice with leukemic cell suspensions confirmed earlier opinions that susceptibility is due to either a single or a double dominant gene (164). Mice normally susceptible to inoculated cells may be rendered resistant by certain treatments (112). Resistance or susceptibility to transmissible neoplasia is probably not governed by humoral factors. Although circulations may be well established between parabions, yet transmission of factors of resistance from a resistant to a susceptible parabion did not occur. Neither susceptibility nor resistance may be conveyed from one parabion to another (59).

Attempts to transmit leukemia from man to animals by blood, urine or emulsions have been unsuccessful. Lymphoblastic leukemia could not be transmitted from a calf to other calves of the same breed (173). Previous irradiation of the spleen of the test animals proved ineffective. Transient leukocytosis occurred, but no other hemopoietic responses were elicited. Urine concentrates of leukemic patients, injected twice daily into guinea pigs, stimulated the reticulo-endothelial system (125, 195). The bone marrow was hyperplastic, the spleen enlarged, and metaplasia of myeloid cells in the liver, spleen, adrenals, and lungs occurred. This causative factor in urine concentrates was not destroyed by boiling. Acetone and ether extracts of spleen and bone marrow of patients with myelogenous leukemia stimulated lymphocytosis (141).

With latent predisposition to lymphomatosis, cutaneous application of carcinogenic tar increased the incidence. A spontaneous lymphomatosis of 2 per cent was raised as high as 50 per cent (23). Injection of bile induced leukocytosis and pathologic states in the liver, spleen, lung, and kidney not unlike those produced by carcinogenic hydrocarbons (119), but immature myeloid or lym-

phoid cells did not appear in the blood. Generalized lymphomatosis occurred in the dilute brown strain of mice on painting with a 0.3 per cent solution of 9,10-dimethyl-1,2-benzanthracene in benzene (105). Hepatomegaly and splenomegaly developed. Hepatic sinusoids were distended with lymphoid cells, and splenic and lymph node architecture was completely lost.

In contrast to mammalian leukemia, fowl leukosis may be transmitted by cell-free plasma or tissue extracts. A complete review of the literature on transmissible fowl leukosis is now available (134a). Filtrable agents have not been obtained in any purified state, owing essentially to their lability. Physicochemical studies (177, 178) indicate that the agent is protein, that the average diameter of the particle is $72 \text{ m}\mu$, and that its molecular weight is 146×10^6 . It is not a pure nucleoprotein, such as the virus of plants, but a much more complex structure. Irradiation with amounts of roentgen rays sufficient to destroy leukotic cells does not affect the virus (93). The infectious agent is localized first in the bone marrow (180), then in the liver and the spleen, where extramedullary foci appear as metastatic centers. The malignant cell in the chicken sarcoma is a fibroblast-like cell (108). Erythrocytes and leukocytes first appear at the site of injection. Monocytes infiltrate and in ten to eighteen hours transform into spindle-shaped cells, which persist and constitute the predominating cellular element, the malignant cell.

Tumor tissue differs from normal in its rate of glycolysis. Elevation in metabolic activity of the erythrocytes has been demonstrated in tumor-bearing animals as in cancerous patients. The curve of glycolytic determinations made on rats after inoculating with virulent uterine tumor showed two phases (200). An initial increase occurred during the first week, followed by a decline, with a subsequent rise after the fourth. Changes in lactic acid were related inversely to the catalase content of the blood. Immunity to transplanted tumor may depend on the state of the reticulo-endothelial system (7). Stimulation of the system by Congo red appeared to inhibit growth of the graft definitely.

Blood changes observed during development of cutaneous tumors in rats after seven to eight months of irradiation include (159): (a) a decrease in red blood cells and hemoglobin, (b) stimulation and depression of reticulocytes, (c) decrease of platelets, (d) decrease in neutrophils, but increase in eosinophils, and (e) an

infiltration of immature forms into the blood stream. Decreases in basal metabolic rate and in blood volume, as well as in erythrocytes and hemoglobin, were observed in rats with fibrosarcoma (15).

PLATELETS

Earlier observations demonstrated a platelet-reducing substance in the spleens of patients suffering from thrombocytopenic purpura. Confirmation of these data has been indefinite. Extracts prepared from spleens of two patients and given to rabbits induced in one instance an elevation and in the other a depression of thrombocyte levels (116). Extracts of spleens of patients with thrombocytopenic purpura were entirely without effect on the thrombocyte level of rabbits (145). Extracts of urines as well as spleens were without effect on the animals' platelet level (184). Data assembled in the laboratory of the Division of Experimental Medicine of the Mayo Foundation¹ were extremely variable and conclusions that such spleens contain some active platelet-reducing substance were not supported. On the other hand, the thrombocyte level in normal rabbits fell from 454,000 to 306,000 in each cubic millimeter of blood following injection of an extract (82).

Thrombocytopenic purpura was produced in the dog by anti-platelet serum derived from rabbits previously injected with canine platelets (185). An acute attack of purpura, lasting forty-eight hours, showed definite thrombopenia, prolonged bleeding, hemorrhage, and edema. Contrary to earlier conclusions, changes in platelets of animals which had received sesame oil for fifty days were not encountered (135). By use of a glass cell connecting a carotid artery with a jugular vein, platelet agglutination time was measured (170). Time relations of heparin on formation of clot and on platelet agglutination indicated that an amount of heparin which would elevate the clotting time to six hours did not prevent agglutination of platelets. Large doses of heparin were required to inhibit platelet agglutination (170). During clotting, changes in the form of the platelet occur. Using magnifications from 7,000 to 12,000, rearrangements in platelet substance, involving appearance of a "granulome" and "hyalome" were observed (205). Fibers, networks, and vacuoles were visible.

Platelets increased for six or seven weeks in animals fed polished

¹ Unpublished data compiled by Dr. A. Uihlein.

rice free from vitamin B (167), but decreases occurred in terminal stages of the deficiency. Abnormal distributions and variability in shape and pattern were common. Treatment restored normal numbers and patterns. Platelets appear to be a source for production of histamine (209). When the total number of leukocytes was depressed by a streptococcal vaccine, no change was detected in the blood histamine. When number of platelets was depressed by anti-platelet serums, blood histamine values were proportionately decreased. The granular series of white cells were thought to contain most of the blood histamine. In dog, cat, rabbit, and guinea pig, marrow content of histamine has been in excess of that found in the blood (30). Leukocytes contain histamine when they leave the marrow (30).

VITAMINS

The relation of the vitamin-B complex to blood levels is not clearly defined. Some substance in brewer's yeast is necessary for blood formation in the pig (204). On a diet of casein, lard, cod liver oil, salt, ascorbic acid, and yeast, pigs became anemic when yeast was restricted. The addition of thiamin, riboflavin, and nicotinic acid did not prevent the anemia, which was characterized by macrocytes, polychromatophilia, and nucleated red cells. The relation of vitamin-B factors to hemopoiesis depends on water intake (63). In pigeons on restricted diets, increased water intake deferred the onset of deficiency symptoms. An increase in platelets and in pathologic granulocytes was seen in rats fed a polished rice diet free from vitamin B₁ (167).

Factor W will not prevent microcytic hypochromic anemia in dogs fed a basal ration supplemented by thiamin, riboflavin, and nicotinic acid. Sixty micrograms per kilogram per day of rat anti-dermatitis factor, vitamin B₆, resulted in marked elevations of erythrocyte and hemoglobin levels (110). Pigeons did not respond to vitamin B₆ (36). Anemia developed in twelve to fifteen weeks in pigeons fed polished rice, supplemented by thiamin. This anemia responded to liver and to yeast, but not to riboflavin, nicotinic acid, or vitamin B₆.

Anemic rabbits recovered normal blood levels more rapidly when riboflavin was provided (44). Almost similar results were obtained when the rat antidermatitis factor, vitamin B₆, was given (44). Thus experimental animals vary in their dependence on the vitamin-B complex. It seems likely that the specific maturation

factor for bone marrow will be quite different from B₂, B₆, or the filtrate factor and it may be found to constitute a distinct and separate fraction of the vitamin.

Chickens fed a synthetic diet, including an alcoholic liver extract supplemented by thiamin and riboflavin, became anemic (83). Total red cells and hemoglobin were decreased, red cells were longer and wider, and volumes were increased. All known vitamins were supplied, and the anemia was not due to inanition. Tentatively, it has been suggested that this factor, antianemic for the chick, be designated as the B₉ vitamin (83).

Thiamin rendered red cells resistant to hemolysis induced by some of the saponins (53). Yeast raised the inhibitory power of the serum. Thiamin induced an elevation in resistance to digitonin hemolysis but was effective only when naturally occurring in the serum. The deduction follows that vitamin B₁ induced a synthesis of some new substance which inhibited hemolysis (53). Vitamins B₁, B₂, C, and E all gave significant increases in the number of reticulocytes. Conclusions were correctly drawn that these elevations represent, not specific maturation principles, but rather results of an irritation reticulocytosis (197). When either A₁, B₁, B₂, C, or D was given to rabbits, changes in the leukocyte counts were recorded (101).

Tests of blood of sheep, pigs, and horses showed that nicotinic acid occurred almost entirely in the red blood cell; there was very little in the plasma (140). For the dog and the pig it is a dietary essential; for sheep and horses its use appears limited. Human blood contains from 330 to 460 gamma of nicotinic acid per 100 cc. of blood (154), while the urine of normal persons contains 50 to 300 gamma per 100 cc. In rabbits, liver contains 7.85 mg. of nicotinic acid per 100 gm. and skeletal muscle 6.47 mg. per 100 gm. (154). Other values for the nicotinic acid content of normal blood range from 0.62 to 0.89 mg. per 100 cc. (150).

Association of scurvy with anemia has long been recognized. When a scorbutogenic diet was fed to guinea pigs (6), four months old, anemia developed in all animals in twenty days. Hemoglobin values dropped from 14.9 gm. to 8.2 gm. per 100 cc.; total erythrocytes fell from 4.5 to 2.2 millions per c.mm. Ascorbic acid prevented the onset of the anemia. There was an elevation in the number of white cells in scurvy (168). Nucleated red cells and abnormal cell types constitute the picture. When diphtheria toxin

was given to guinea pigs, animals withstood the effects far more satisfactorily if the vitamin-C supplements were adequate.

Likewise, ascorbic acid in doses of 0.2 gm. increased the total number of erythrocytes (33), and stimulated the reticulo-endothelial system (60), without any apparent effect on leukocytes (33). If anemia was induced, an even greater stimulation of the erythrocytes, reticulocytes, and nucleated red cells occurred. The injection of 100 to 200 mg. of ascorbic acid into rabbits (28) increased the total number of leukocytes by 2,000 cells, an elevation perhaps not significant. Nicotinamide and cortineurin induced a very rapid rise in the hemoglobin and erythrocyte levels in postinfectious anemias and in the anemias of prematurity (61).

Avitaminosis A was accompanied by mild leukopenia, decrease in granulocytes, and increase in the large lymphocytes and in myelocytes (1). These conditions reverted to normal when adequate vitamin A was provided. Diets deficient in vitamin A produced in rats a gelatinous degeneration of the bone marrow (1), often replaced by fibrous tissue. Deprivation produced a diminution of thrombocytes (4) ranging from 31 to 69 per cent. Atrophy of marrow with significant decreases in eosinophilic and megakaryocytic cells occurred.

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HEART¹

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PHYSIOLOGICAL PROPERTIES

The beginnings of the embryonic heart beat are being studied with increasing frequency by tissue culture methods. Rabbit embryos nine days old cultured in hanging drops show independent tempos and different patterns of beat in the two primitive lateral tubes. Until fusion occurs, no atrial contractions are evident (57). In rat embryos, contractions occur before fibrillar differentiation (82). Heteroplastic heart grafts of *Amblystoma trigonum* to *A. punctatum* retain their intrinsic rates and types of growth during development (42). Other reports concern the effects of osmotically active substances (211), of ions (39), and of constant currents on rhythm (150). In molluscs, acetylcholine decreases systolic and diastolic size (120). Urea augments the amplitude of contraction of the perfused heart (175); bile acids and salts cause slowing, diminished amplitude, alternation, and extra systoles in perfused rabbit hearts (243).

The anatomical pathways by which impulses spread from the sinoatrial node toward the ventricular conduction system remain uncertain. According to Pannier (171), the sinoatrial node contains small A cells and larger peripheral B cells which connect with subendocardial Purkinje tissue of the atria. A bundle similar to that described by Thorel in 1908 is recognizable in the cat's heart.² Other workers (186) found that strips of left atrium apparently devoid of specific muscle not only contract but are capable of various conduction anomalies. The effects of crushing the atrioventricular bundle are redescribed but no new facts are revealed (119). Katz and associates (34, 154) tested atrioventricular conduction in dogs by maintaining a perpetual atrial fibrillation and counting the number of ventricular beats. They found that

¹ This review covers the literature published between July 1939 and August 1940.

² Note added to proof: See also, D. J. Glomset and T. A. Glomset, *The American Heart Journal*, 20, 389 (1940).

atrioventricular conduction is enhanced by epinephrine, paradrine hydrobromide, barium chloride, and atropine; it is depressed by acetylcholine, mecholyl, and quinidine. Enhancement followed by depression of conduction resulted from asphyxia, anoxia, and hypercapnia. The question has been raised as to whether the method is a test for atrioventricular conduction or for the number of impulses that bombard junctional tissues.

Much scientific and practical information is still derived from a study of the reaction of the ventricles to stimuli and drugs. Perhaps a distinction ought to be made between excitability, the elicitation of a reaction by environmental change, and responsiveness, the modification of regular activity by environmental influences, such as drugs (30). Drury has previously (in 1926) defined the "effective refractory period," as the time interval preceding the earliest moment at which an impulse, of sufficient intensity to be conducted, can be liberated after a previous excitation. This period is not changed in hypertrophied ventricular muscle of rabbits, a fact indicating that any functional disability is not reflected in the refractory state (53). Since the early experimental observations of Kronecker & Marey, there have been periodic claims that the ventricle is not refractory throughout systole. Most of the evidence has been spurious, the results being due to current spread and re-excitation of the ventricle by a premature atrial beat. New evidence is offered (202) that the ventricles of turtles and bullfrogs, like those of the dog (250) respond to a strong stimulus during the latter portion of systole. The occurrence of ventricular complexes—frequently starting before the next P wave—and the preservation of the natural sinus rhythm preclude an atrial origin of the premature ventricular beats (247).

Strong condenser and induction shocks applied late in systole to a minute area of the myocardium during the so-called "vulnerable period," lead to fibrillation of the dog's ventricle (252). Shocks applied at any other time of the cycle are ineffective in this capacity. A single sine wave or a rectilinear shock of sufficient strength and lasting .01 to .03 sec. is likewise effective during the vulnerable period (248, 253). Finally, a mechanical prick, a stab, or lodgement of a bullet (213) may induce ventricular fibrillation. A 60-cycle alternating current of more than four or five waves, i.e., exceeding the duration of the vulnerable period, always evokes fibrillation when the waves start during the vulnerable phase and are strong

enough. When they fall entirely during diastole, they cause one or two premature beats but fibrillate the ventricles when the last of such a wave train strikes the vulnerable period of a premature beat (248).³

A number of investigations are concerned with the sensitivity of the ventricles to fibrillation and with agents which increase or decrease their reactivity. Most of these involve test of agents which prevent or minimize the tendency of the ventricles to fibrillate when epinephrine is administered with chloroform, benzol, or cyclopropane. Shen (203) reports on the effectiveness of procaine in preventing benzol-epinephrine fibrillation. Orth *et al.* who had previously reported that epinephrine-chloroform mixtures are not dangerous in dogs (170), but that epinephrine-cyclopropane is, have continued these studies on related amines. Primary and secondary sympatheticomimetic amines having 3,4-hydroxyl groups on the benzene ring are particularly dangerous when used with cyclopropane. Cyclopropane-epinephrine irregularities and fibrillation are prevented by anemic decerebration, which suggests a central action anterior to the pons (7). Other researches indicate that chloroform-epinephrine and benzol-epinephrine fibrillation are preventable by use of caffeine and amyl nitrite (44), that F-933 protects against electrical fibrillation of the ventricles in cats and rabbits (51), and that cyclopropane-epinephrine fibrillation is prevented by procaine and less certainly by *p*-aminobenzoic acid, and the calcium double salt of benzylsuccinic acid (31, 149). While all of these results are suggestive, a more critical criterion as to the vulnerability of the heart to various agents is needed. We (253) have suggested a method for evaluating the fibrillation threshold quantitatively by the strength of a brief rectilinear shock which just induces fibrillation when applied during the vulnerable period of a beat. According to this criterion, procaine raises the fibrillation threshold somewhat and myocardial ischemia reduces it significantly. Determination of the quantity of barium chloride which just produces fibrillation in the ventricle of the perfused heart of the cat, indicates that quinidine raises the fibrillation threshold (20). Nahum & Hoff (163) induced atrial fibrillation by localized application of acetyl- β -methylcholine.

³ Note added to proof: See also, R. Wégria and C. J. Wiggers, *American Journal of Physiology*, 131, 104, 119 (1940).

INNERVATION OF THE HEART

The distribution of nerves to the heart and their *modus operandi* continue to be studied. Nonidez (166), by a differential staining technique, traced parasympathetic postganglionic fibers to the nodes of the conducting system, arteries and muscles of the atrium, and a few to the ventricles in dogs. The middle accelerator nerve of the sympathetics supplies chiefly the ventricles; the inferior cardiac nerve carries mainly afferent fibers; the superior cardiac nerve ends chiefly on large arteries and does not reach the heart. Fibers of both systems terminate separately and not as a common "terminal reticulum." Substances acting on the autonomic nervous system alter the refractory period and chronaxie of the fish (10) and frog (71) heart. Vagal stimulation increases the rheobase and shortens chronaxie of the atrium, but the threshold for induction and condenser stimuli is not increased. Accordingly, the vagus may be said to decrease irritability through increased accommodation to stimulating currents (75). Barbiturates paralyze postganglionic vagus fibers of the frog's heart (95), as had been previously shown for vagus fibers in dogs. Evidence has been periodically presented that the vagus contains parasympathetic accelerator fibers. Further evidence for this has been reported (28). Kabat (121) obtained cardiac acceleration through stimulation of intracranial vagus rootlets. The fibers are carried predominantly in the right vagus. They are not reflexly excitable either from the carotid sinus or sensory vagus, but react to central stimulation (specifically, to anoxemia). It is concluded that they do not participate in cardiac acceleration during exercise or emotional states.

The finer reflex adjustments of cardiac rate have received additional study (235). Cardiac acceleration due to fall in blood pressure is not due entirely to carotid sinus and aortic reflexes; it occurs also in the denervated heart (256). Its assignment to increased adrenal secretion would have been more convincing had it been shown that the effects are absent after excision of these glands, as was done in the case of intestinal reactions. The observations that acetylcholine can produce primary and secondary effects on cardiac rate are important in evaluating blood pressure changes which are customarily attributed wholly to vasomotor actions (see also 49).

The reflex regulation of heart rate appears to be somewhat

specialized (28). According to Comroe (41), the aortic chemoreceptors do not affect the cardio-inhibitory center reflexly. Additional evidence is offered that amplitude of the pressure pulse as well as mean pressure affects vasculocardiac reflexes (224). Decrease of pressure in the left atrium is said to have a reflex pressor action opposing the depressor vagal reflex (242). The sympathetic cardiac nerves may be excited centrally by high intracranial pressure. Indeed, the claim is made that the rise of blood pressure which occurs is due solely to accelerator action and increased ventricular contractions. In denervated hearts, reflex secretion of epinephrine causes similar effects (66). Reflex slowing of the heart and vasodilation following administration of veratrine is said to be due to afferent impulses arising in the ventricle and carried by the vagi (113). Cerebral anoxia is believed to abolish the normal reciprocal action of centers controlling the heart rate; but the proof for this conclusion is not quite clear (106).

Two reports are of interest in regard to the interpretation of cardiac pain. King (127) found several histological types of nerve endings, interpreted as stretch and pain receptors, in the myocardium of the rat. Maison (147) presents evidence from studies on skeletal muscle that ammonium, potassium, or sarcolactic ions are not the P factor (of Lewis) in ischemic pain.

ENERGETICS AND METABOLISM

With the exception of several excellent reviews (122, 241, 261, 272), little new work has appeared in the past year. Repetition of experiments on various types of isolated failing preparations with various improvements of technique continues to show that, in Chicago, dogs' hearts fail because of a gradual diminution in the total amount of energy released (123), whereas, in Minneapolis, dogs' hearts fail due to decrease in the amount of work developed per cc. of oxygen consumed (241). Several investigators (70, 182), however, consider the heart-lung preparation unsuitable for the study of problems dealing with the external work of the heart. Under certain circumstances, a positive error equal to one cubic centimeter for each 10 mm. increase in mean aortic pressure may be incurred in quantitative estimation of diastolic volumes by means of a cardiometer (158). In experiments on isolated cat hearts perfused with hemoglobin-saline, it was concluded that a specific oxidation-augmenting effect of epinephrine is not demon-

strable (70). The effects of vitamin-B₁ deficiency of the heart are reviewed in a recent monograph (271). A few additional references may be mentioned (18, 100, 145, 176). The effect of the deficiency is probably due to a defect in metabolism rather than to a toxic effect of circulating metabolites (100, 145).

CARDIODYNAMICS

The method of O. Frank for determining volume-tension relations of the frog's ventricle, arranged to contract isometrically, isotonically, and auxotonically has been utilized again in studying the dynamics of the frog heart (181). Among the interesting conclusions are the inferences that, at constant diastolic volume, the pressure at the end of systole determines the extent of ventricular emptying, and that fatigue and acetylcholine abbreviate contraction. Recent studies (202) demonstrated that pressure relations in the ventricle and aorta of bullfrogs and turtles display all the characteristics shown on similar records from mammals, including the presence of an isometric period and incisura. It appears that these ventricles do not empty as well as those of mammalian hearts during systole. Systolic aortic pressures range from 26 to 34 mm., diastolic pressures from 12 to 18 mm., giving pulse pressures of about 15 mm. Hg. The intact ventricle responds to increase in initial tension and length by increase in output and lengthening of systole. Increase in rate above 30 or 40 per min. induced by electrical stimulation of an atrium was accompanied by a reduction in aortic pressures. A low optimum rate for cold-blooded hearts was suggested (202). Further studies on strips of contracting ventricle and on patients strongly suggest that digitalis in moderate therapeutic doses acts by increasing the systolic tension development for a given diastolic length and that the slowing of the heart observed represents largely resumption of vagal tone with improvement of the circulation (78).

Cardiac output.—New estimations of cardiac output in health and disease have been reported by the acetylene rebreathing method (80, 195, 199) which has undergone some modifications (2), by the physical methods of Broemser and Bazett (47, 199), by the ballistocardiograph (217), by the roentgenkymograph (126), by the dielectrocardiograph (185) and impedance cardiograph (169), the two latter being both designated as radiocardiographs. Data from normal resting adults (47, 126, 169, 195, 199, 217, 263)

continue to indicate that the normal stroke volume ranges around 60 cc. Contrary to many previous reports on man, but consonant with cardiometric observations on dogs, the human stroke volume increases significantly in extreme bradycardia from 57.5 cc. to 90.1 cc. (126). It is claimed that, both in normal patients and in those with cardiovascular disease, cardiac output is not affected by posture (80). However, cardiac output apparently decreases greatly on standing in subjects recently placed in a warm environment after being acclimatized to a cold one. This is thought to be related to the reduction in blood volume found in subjects so acclimated (32, 199).⁴ Physical training decreases the heart rate and increases the stroke volume (as determined by the acetylene method) both at rest and during equivalent exercise (195). The minute volume per unit of surface area (195), the arteriovenous oxygen difference, circulation time, venous pressures, arterial pressures, cardiac size, and oxygen consumption are not significantly altered by physical training (223).

The effects of drugs and chemicals on cardiac output continue to be studied both in animals and man. In anesthetized dogs, carbon dioxide (up to 10 per cent) decreases systolic size and increases diastolic size and stroke volume measured by cardiometers. Higher concentrations decrease the stroke volume as does chloroform, but the depressant effects of the latter are reduced by administration of high concentrations of oxygen (43). The stroke volume is increased by xanthine drugs in anesthetized animals (36), also by neosynephrine (126) and epinephrine in man (47). The effects of the latter resemble those of exercise (47). Optical records of pressure pulses and velocity curves in the thoracic aorta indicate that xanthine drugs, epinephrine, asphyxia, etc., increase the pulse pressure, the mean flow, and peak velocity during systole; pitressin has opposite effects (87).

Pathological physiology.—In clinical patients it is reported that cardiac output is reduced (a) during pneumothorax (220), (b) in compensated heart disease, and even more during decompensation (221, 263), and (c) in ambulatory patients subject to various cardiovascular disorders (214). The cardiac work per unit volume of heart also is reduced in congestive failure (215). Studies on a double-heart circulation model led to the conclusion that simple

⁴ Note added to proof: See also, E. Asmussen, *American Journal of Physiology*, 131, 54 (1940).

weakening of one or both sides of the ventricles does not produce the pressure relations which are found clinically in congestive failure (216). The operation of some extracardiac force capable of raising static blood pressure, particularly in the veins, was postulated. The concept is supported by the existence of elevated venous pressures in subjects that died of congestive heart failure (216). A better approach to an evaluation of regurgitant volumes and the degree of valvular efficiency in human valvular insufficiency was made by Keys *et al.* (125), who estimated regurgitant volumes by the difference between stroke volumes obtained by roentgenkymography and by rebreathing acetylene. In mitral leaks, valvular efficiency averaged 52.1, 69.7, and 86.9 per cent respectively in cases diagnosed as severe, moderate, and slight regurgitation. In compensated aortic regurgitation the valvular efficiency ranged from 42 to 84 per cent. Further evidence is presented that opening and closing arteriovenous fistulae in experimental dogs respectively increase and decrease cardiac output, determined by the Fick method (153). Failure of the right ventricle resulting from conditions leading to high right ventricular pressures is at least partly explained by diminished blood supply and relative anoxia of the right ventricle (159, 240).

Several procedures have been refined for studying cardiac activity in the closed chest of man. Simultaneous recordings of cardiopneumatic movements and central arterial pulses lead to the conclusion that the reduction in cardiac output during the Valsalva experiment is caused by venous obstruction rather than by impeded pulmonary flow (162). Taquini (230) redescribed optically recorded esophograms in various regions of the esophagus and re-emphasizes their value in the interpretation of mitral lesions. Roentgen-ray visualization of the heart and intrathoracic vessels by diodrast is proving safe and useful clinically in measuring the size, shape, and location of individual chambers of the heart, in determining the site and degree of structural abnormalities, in calculating cardiac weight, volume, and thickness of walls, and in evaluating circulation time in the central vessels (183).

Herrmann & Decherd (103) review the more important theories of cardiac hypertrophy. Clinicopathological correlations of predominant hypertrophy of right and left chambers with existing pathological conditions continue to be reported (69, 200, 225), but give no additional clues as to the mechanisms concerned. Nor has

any significant advance in the solution of the problem been made experimentally. The difficulty of producing experimentally a degree of hypertrophy, comparable to that seen clinically in man, continues. Failure to induce hypertrophy by stretching the hearts of rats and rabbits by large infusions of acacia solution led to the conclusion that temporary dilatation is not the agency causing hypertrophy as claimed by the Horvath-Eyster hypothesis (103). On the other hand, hypertrophy was successfully produced by placing snug silk ligatures, which caused no initial constriction, around the pulmonary artery or aorta in very young puppies (108).

In both clinical and experimental cardiac hypertrophy the ratio of capillaries to fibers is the same as normal animals but, because of the increased cross-sectional area of the fibers, the number of capillaries and the total capillary surface per cubic millimeter of myocardium are decreased. These facts and the finding that the heart normally utilizes around 75 per cent of the oxygen content of its blood supply lead to the suggestion that at some stage in the course of hypertrophy of the heart a point will be reached where metabolic exchange will be interfered with (245, 273).

Pericardium.—Various functions have been attributed to the pericardium; among them are (a) no function, (b) protection against overdistention, (c) creation of a diastolic support or an initial reserve force aiding in contraction, (d) exertion of a suction force during relaxation, (e) co-ordination of mechanical contraction of the two ventricles, (f) maintenance of the heart in a relatively fixed position, and (g) creation of smooth gliding surfaces. The experiments of Barnard (1897) are frequently quoted in support of the accepted view that the pericardium is practically inelastic, although it is realized that it must enlarge somehow in extreme forms of cardiac hypertrophy. Histological and mechanical studies of pericardial tissue in dogs and cats (165) now lead to the conclusion that the pericardium has both elastic and plastic properties, the latter being due to the presence of collagenous fibers. Experiments in which radiopaque fluids placed in the pericardial cavities of dogs and cats were completely expelled from a cannula during several diastoles led to the conclusion that the ventricles completely fill the pericardium (164). This is difficult to reconcile with former observations (65) that 21 to 79 cc. of saline solution can be accommodated without affecting venous pressures and presumably the filling of the ventricles. Furthermore, space

must be available to accommodate the normal heart when it enlarges as a result of extreme slowing.

The conclusion that cardiac tamponade reduces cardiac output by impeding ventricular filling is probably correct, but new evidence supposed to support this is not convincing (236). Elevation of pericardial pressure by injection of air, in a patient with pneumopericardium, caused mainly a rise in venous pressure which maintained itself at a level about 50 mm. of water above the pericardial pressure. Above a critical level of 145 mm. of water in the pericardium, this difference became progressively less; the arterial pressure declined and circulation time increased, despite acceleration of the heart (3).

HEART SOUNDS

The monograph of Orias & Braun-Menendez (265) and its English translation (266) have reawakened interest in the usefulness of heart sound registration, both for physiological and clinical purposes. They present evidence that four sounds are discoverable in the majority of normal adults and enunciate the concept that many well-known auscultatory signs in disease (duplication of sounds, gallop rhythm, etc.) do not arise *de novo*, but are merely exaggerations of inaudible components present in the normal heart. Further studies have been made on medical students (24) using taut membranes with higher frequency and greater damping, thus eliminating adventitious vibrations arising in the chest and perhaps in loose membranes. Two aspects of phonocardiography which are applicable to any system were emphasized: (a) The character of heart sound records is not only a function of the physical constants of an apparatus but also of the way it is used and adapted to any particular subject. In all types of recorders there is danger from the two extremes of recording too little or too much. (b) A continuous stream of irregular vibrations arises in the chest due to the motions, contacts, and recoil of the heart and large vessels, and to the filling or emptying of the cardiac chambers. Records should be as free from such vibrations as consistent with full reproduction of the real sound waves. Following these principles, it was found that atrial sounds occurred in 22 per cent of normal medical students; a third sound was observed in 4 per cent when seated and in 26 per cent when lying down. The recorded first sounds varied considerably as regards configuration and duration

but could be roughly placed in four categories. The apical second sound, composed of a few decrementing vibrations and generally considered typical of normal adults, occurred in 72 per cent of cases; in 8 per cent the sounds were bizarre, irregular, prolonged, or duplicated, while in 20 per cent they were louder than the first (24). In somewhat overfiltered records, Sacks & Roberts (189) found an incidence of 33.3 per cent of third sounds, with occasional atrial sounds. Atrial and third sounds are apparently not as common in adult males as the reports of the Argentine investigators indicate.

The cause of the heart sounds is of continuing interest. They have been assigned solely or conjointly to vibrations caused by muscular contraction, closure of valves, or multiple cardiac structures. Recently, Dock (50) and Lewis & Dock (140) concluded that no first sound occurs when the atrioventricular valves are rendered functionless in experimental hearts. This was not confirmed by recent studies (76, 205). The possibility of separating low frequency vibrations—below or near the lower auditory limit—has been attempted (48, 76, 205), but no great field of usefulness can be foreseen for such studies. It cannot be emphasized too often that sounds (and murmurs) originating in the heart are modified not only in their transmission to the chest wall but by the tubing and endpieces of the stethoscopes commonly employed. A recent restudy of stethoscopes (118) indicates that the endpiece should be of adequate size (about 3 cm.) and have a shallow chamber with the leading tube of small diameter. Diaphragms of endpieces act as filters for lower pitched vibrations, but only when they are rigid. Contributions, too numerous to record, have been made on various clinical aspects of phonocardiography. Among these are studies on fetal sounds (267) and those of new born (143).

ELECTRICAL PHENOMENA

Are bioelectric currents fundamentally an expression of the depolarization of transversely polarized membranes or are they due to longitudinal movement of contiguous bipoles (Craib)? This question continues to be actively studied in the heart. Ashman *et al.* have made a number of reports (8, 9, 12) of data which they believe difficult to harmonize with the membrane theory and which favor Craib's view that electrical variations travel as if the dipole were oriented longitudinally: (a) Monophasic curves are derivable

from an uninjured region and a cooled apex (8). The latter is apparently not considered in the nature of an injury or depolarization phenomenon. (b) A lead from a strip of muscle and a point 14 cm. distant in a saline bath showed that a positive variation preceded the negative deflection when the strip was stimulated, but the order of polarity of the final (T) wave was the opposite (12). (c) Successive leads from two points (A-B) on a heart-muscle strip and from a saline bath (C) showed that the positive potential was not simultaneous in leads A-C and B-C, as would be the case if the positive variation were due to reduction of potential at C. No plateau occurred in lead B-C. The magnitude of the positive phase was less in lead B-C than in A-C (9). Despite meticulous execution of experiments, such observations may not be conclusive. Harris (98) confirmed the previous observations of Bishop & Gilson that the character of similar leads from an intact turtle ventricle and a saline bath can alter their initial polarity, this depending on the placement of the exploring electrode. It is not impossible, in such preparations, that the potential of the exploring electrode is sometimes dominated by local changes, and at others by the field potential (227).

The question as to the type of lead which gives the most exact information with regard to incidence of punctate negativity on the cardiac surface is being restudied with great care in several laboratories. Goldberg & Eyster (79) found an intimate relationship between initial changes in electrical potential and onset of shortening in various regions of the turtle ventricle (the differences amounting to .05 sec. or less). The onset of contraction synchronized with the main peak of a differential lead (Garten) and fell during the interval (which lasts .04 sec.) between the first positive and second negative peak of a unipolar lead. The differential curve is regarded as a first derivative with respect to time of the unipolar curve (according to a personal communication). The electromechanical relations of the dog's ventricle are similar (64). However, Harris (98), using modified Garten electrodes consisting of two contiguous points, found some differences in the relation of the peak to deflections of a standard electrocardiogram when the electrodes were rotated, but the relation to the onset of the first negative deflection of the differential curve was more constant.

The nature of the sequential changes following injury justly command attention since they are basic to interpretations of mono-

phasic leads directly from the heart and indirectly from the body. Decker (45) concludes that conduction of impulses is important in genesis of monophasic action potentials. That an injured region on the turtle ventricle not merely drops to zero potential but actually becomes positive with respect to resting muscle was confirmed (9). The origin of the action potential in skeletal muscle is discussed by Sugi (227). The method of Craib for demarcating field potentials is criticized on two counts: (a) Craib mapped equipotential lines only in distant regions, and (b) he considered an equipotential line running parallel to the injured surface through a normal part of the muscle as a line of zero potential, which is not allowable since "zero" is undeterminable. Nevertheless, using this procedure, slightly modified, Sugi reaches the following conclusions: (a) The potential measured on a muscle is often the field potential. (b) The uninjured surface of an injured muscle is not, as is claimed by the membrane theory, the source of the injury potential; it is generated at the injured surface. (c) The distribution of an injury potential in the surrounding field depends on the extent of injury and on the physical conditions of the field medium.

Katz and his associates (226) employing unipolar leads also found that an injured area became negative during diastole and positive during systole with respect to a limb potential, but explain the changes on the membrane theory. As a corollary, monophasic curves from an injured and uninjured spot still measure the duration of negativity at the exploring electrode, but not the true onset of negativity. The course of events within and around an area injured by injection of 95 per cent alcohol were also studied (226). From enlarged records three-dimensional time-space models were made. They display mountains and canyons of electrical potentials, indicating that the impulse first spreads aberrantly in and, later, around the injured area; that depression of S-T is maximum in the injured area; that the coronary type of T wave (in unipolar leads) is restricted to the periphery and environment of the injury; and that it does not develop tardily after injury. Conclusions with regard to standard leads are that changes in QRS during injury are due to altered spread of impulses in injured territory; that the T-Q elevation and S-T depression are caused by partial repolarization of the injured area during rest and irresponsiveness during activation (possibly of the rest of myocardium); that the upright coronary T is due to responses of the partially in-

jured region in which polarization is delayed. Other interpretations of the monophasic or "high take off" in conventional leads have been offered (46, 197). Clerc & Quinquaud (38) cut a dog's ventricle transversely, after which basal and apical portions beat at different tempos. When the apex was removed, a monophasic curve was recorded (limb leads); when it was replaced, two oppositely directed monophasic curves were obtained at different times. When the two parts occasionally beat simultaneously, a summated diphasic curve resulted. The reviewers marvel at the experimental achievement of keeping two sections of the exsanguinated heart beating but leave the implications of the experiments to the reader.

We may summarize the year's progress on the nature of cardiac action potentials by stating that many exquisite pieces have been added to the jigsaw puzzle, but not enough have yet been fitted together to reveal the final nature of the picture which will develop.

The human electrocardiogram.—A simplified interpretation of the standard leads, including changes due to infarction, has been presented (132). The desirable nomenclature and terminology of electrocardiograms was subjected to democratic discussion in fascist Germany (246). Use of the electrocardiogram to detect variations in force of ventricular beat is defended by arguments that are somewhat strained (112). Science is truly advancing when changes in ventricular vigor can be detected by a few microvolt variations in currents derived from needle electrodes thrust into a skeletal muscle! Monocardiograms from normal students have been analyzed (37), the importance of stature and body type being stressed. Studies have been made of electrocardiograms from normal subjects (109, 111, 116, 151), including the U wave (172), and discussion of peculiarities occasionally found (249). Other papers deal with evaluation of conduction times (94, 190), variation of initial R waves (6), the significance of Q_s (13, 239); the validity of the Einthoven triangle (196), the significance of electrical axis deviation (40, 110, 129), the electrocardiogram in coronary disease (73), and changes in the aged (137, 191, 244). The effects of posture (173, 219), exertion (131, 204), and athletics (58, 104, 105) are again analyzed.

The effects of epicardial and deeper myocardial injuries due to mechanical and chemical agents are compared in several communications (23, 128). Superficial injuries—possibly by reduction of good conduction surfaces—cause greater deformation of the S-T

segment than deeper ones. Small surface injuries may cause considerable distortion. This may explain the significant alterations found in pericarditis, a disorder which has received considerable attention (68, 168, 238).

Electrocardiographic changes have been studied in hypertension (138, 179), nephritis (254), anemia (229), aneurysm (167), pulmonary embolism (207), clinical and experimental hyperthyroidism (11, 27, 60, 210), pregnancy (184), pneumoperitoneum (59), pylorospasm (141), gastric distention (115, 161), and cholelithiasis (187). The effects of menopausal disturbances and ovarian hormones (4, 192), and of vitamin-B₁ deficiency (56, 156, 208, 271) have been discussed. Boyle *et al.* (25) studied electrographic changes due to diphtheria in dogs, which included changes in the T wave and block. Of interest are the electrographic changes which occur during heat stroke (155) and fear of operations (146). Other articles deal with the effects of oxalic acid hyperglycemia (5), asphyxia (133), decreased barometric pressure (17), caisson disease (26), carbon monoxide poisoning (180), tobacco smoking (16, 19, 116), veratrine (206), and various ratios of potassium to calcium (35, 55, 107, 232). In the field of neurology, articles on electrocardiography include those pertaining to familial periodic paralysis (222), progressive muscular dystrophy (178) and shock therapy (15, 81, 136, 209).

The topography and temporal incidence of electrical potentials over various regions of the thorax have been studied systematically by a number of cardiologists (22, 61, 67, 72, 93, 97, 177, 198, 201, 219). A more critical sifting than is warranted for this occasion should prove valuable to clinicians in the interpretation of chest leads. Analysis of precordial leads in angina pectoris (157, 255) and myocardial infarction (21, 77, 130, 142, 188) are too numerous to catalogue completely.

CORONARY CIRCULATION

Anatomy.—Further studies have been made of the injected unrolled heart by roentgenradiography, by microscopic studies, and by dissection. They emphasize the shortness of the common left coronary in the dog (29, 174). While demonstrating the essentially terminal character of the normal coronary arteries in man and the dog, they reiterate the presence of many latent anastomotic communications less than 40 μ in diameter (174, 194) between the main

rami and between the arterioles and both ventricular cavities (273).

Lymph.—The entire lymph drainage of the heart may be collected from a channel emptying into the cardiac node which lies between the innominate artery and the superior vena cava close to the aorta. The lymph pressure is around 15 cm. (of lymph) and the rate of flow follows the mean blood pressure (52).

Blood flow.—The phasic changes in coronary flow have been recently reviewed (88). With constant circulatory conditions, inflow of blood into a coronary artery, measured by an optically recording constant-pressure flowmeter, was roughly proportional to the differential pressure (the difference between perfusion pressure and peripheral coronary pressure) over a range of 150 to 20 mm. Hg (85). Registration of the inflow from the aorta into a coronary artery by means of an optically recording orifice meter indicates that, in the left anterior coronary artery, the rate of inflow abruptly diminishes during isometric contraction, accelerates during the rise of aortic pressure, diminishes to a rate of flow less than the diastolic during the latter half of systole, and again increases with the onset of isometric relaxation to a maximum about mid-diastole (91). Similar fluctuations of flow occur in the right coronary artery, but the minimal rate of systolic flow is more nearly equal to the minimal rate of diastolic flow (88). While the rate of inflow is modified by volume-elasticity and compression of vessels, the rates of inflow at the end of diastole and near the end of systole—that is, at the peak of the peripheral coronary pressure curve—probably indicate the magnitude of diastolic and systolic intramural flows respectively (91), and may be used as a criterion of vasomotor changes.

Factors affecting coronary flow.—An analysis of factors affecting coronary flow requires both synthetic and analytical methods of approach. A recent review (114) covers many phases of this problem. Accurate determinations of myocardial pressure during the heart cycle would elucidate greatly our knowledge of the control of coronary flow by the beat of the heart. This has not yet been achieved. Partly because of elastic distortion of the tissues by a balloon, pocket of fluid, arterial segment, or other device, measurement of the intramyocardial pressure is unsatisfactory as regards the absolute magnitude of the pressure (89).

In the heart-lung preparation, the mean rate of flow measured by the thermostromuhr was roughly proportional to the mean

aortic pressure (102).⁶ However, in the whole animal, as the aortic pressure was increased, the ratio of the diastolic intramural rate of flow to the simultaneously existing aortic pressure decreased, indicating an increase in the resistance to flow (86, 90). In the heart-lung preparation, increased cardiac output had no effect on mean coronary flow (102). In the anesthetized whole animal preparation, systolic and diastolic intramural rates of flow were elevated and the ratio of intramural flow to pressure in diastole was consistently increased, a fact indicating a decreased resistance to flow (86, 90). These differences in the effects of cardiac output may have been due to the absence of innervation in the heart-lung preparations. Again, the mean rate of flow increased with rise in heart rate in the heart-lung preparation (102), whereas opposite effects were induced in the systolic and diastolic intramural rates of flow and of total flow in intact animals (88). Decrease in the apparent resistance to flow in both systole and diastole resulted from decreasing the viscosity of the blood and followed restoration of flow after a short period of ischemia (86, 90). By using the above methods, earlier conclusions based on differential pressure studies (83) regarding the effects of aortic valve lesions were substantiated (86). Cardiac venous and peripheral coronary arterial pressures were elevated almost to aortic levels (233), and coronary artery inflow was reduced immediately after ligation of the coronary sinus or great cardiac vein (88). After twenty to thirty days the pressures returned almost to normal levels (233).

Further evidence has been obtained that elevation of the pulmonary arterial pressure can reduce thebesian flow (159). However, when pulmonary resistance is increased peripherally by an unnatural "Starling resistance" instead of by central pulmonary stenosis, the difference in mean aortic and pulmonary pressures must be less than 40 mm. Hg before effects can be detected by rough outflow measurements. Without registration of optical pressures, it is impossible to decide whether the rise of pulmonary mean pressure in the preparation used was accompanied by significant elevation of systolic pressure in the right ventricle, a requirement for impedance of thebesian flow (117). In any event, these experiments demonstrate that an elevation of mean pul-

⁶ Note added to proof: See also, E. Hausner, H. E. Essex, J. J. Herrich, and E. J. Baldes, *American Journal of Physiology*, 131, 43 (1940).

monary pressure in isolated hearts does not necessarily influence the partition of coronary flow.

The possibility of functional communications between the right ventricular cavity and the myocardial capillaries was recently studied by keeping separate the blood entering the left and right ventricles and adding dye to the latter. With normal pressure relations no communication could be found, but, when right ventricular pressure was elevated above that of the left ventricle, the dye colored both ventricles and was found widely distributed throughout the capillaries (273).

Nervous control.—Further evidence of a nervous reflex control of the coronary circulation was provided by the registration with the thermostromuhr of a decrease of coronary flow in fifteen out of twenty-one dogs following distension of the stomach or peritoneum, a decrease which was abolished or converted to an increase by atropine (74). However, the exact part played by the various vasomotor nerve pathways and chemical intermediaries in controlling coronary flow is still not satisfactorily established. The only new observations with regard to the vagi are the above effects of atropine and the observation that in similar preparations, acetylcholine increased the mean coronary inflow (63). With regard to the sympathetics, it was observed that (a) stimulation of cardiac branches of the stellate ganglia and intravenous injection of epinephrine increased the mean coronary flow measured with the thermostromuhr in heart-lung preparations, even when the mean blood pressure remained constant (102); (b) increased total flow and ratio of diastolic flow to differential pressure followed stellate stimulation in the anesthetized dog (88); and (c) in experiments on isolated fibrillating and quiescent cat hearts, an initial momentarily increased flow was recorded, which was considered to be relatively greater than the initial increase of oxygen consumption (70). An error may have been introduced in the last experiments by the considerable distance of the "venous" photovoltaic cell from the heart. The existence of an adrenergic or sympathetic coronary dilator action was deduced from these experiments (70, 102). However, intracoronary injection of pure epinephrine in the anesthetized whole animal, in doses small enough not to modify aortic blood pressure, neither significantly increased nor decreased diastolic intramural flow, measured with an optically recording orifice meter, despite evidence of myocardial

stimulation. With larger doses, the increased rate of diastolic intramural flow was often not greater than the elevation of aortic diastolic pressure. The latter results indicate that epinephrine does not cause any definite coronary constriction, and that a coronary dilator action is not prominent and may be secondary to release of metabolic products (84).

Drugs.—Further evidence of the coronary dilator effects of the xanthines, nitrites, histamine, muscle adenosinephosphoric acid, thyroxin, papaverine, atropine, and sodium pentobarbital, and of the coronary constrictor effect of pitressin, was obtained (*a*) in the fibrillating dog and cat hearts (114); (*b*) by measurement of the mean coronary sinus outflow (134); (*c*) by registration of peripheral coronary pressure (84), and of the instantaneous rate of inflow into the left coronary artery (84) in the anesthetized dog; and (*d*) by thermostromuhr records of mean coronary artery inflow in unanesthetized and anesthetized dogs (62, 63). Twice daily injections of papaverine caused no reduction in the size of infarcts experimentally produced in dogs (135). However, the xanthines and nitrites minimized the electrocardiographic changes and prolonged the time for appearance of angina induced by exercise in patients (139). Intravenous glucose injections did not diminish the pain or the electrocardiographic changes during angina induced by exercise in patients (193), while in perfused hearts, any increases of coronary flow associated with the addition of glucose to the perfusion fluid were due to the osmotic effect of the hypertonic solution so produced (144).

Experimental occlusion.—Experimentally, gradual occlusion of the coronary artery has been produced in several ways: by successive tightening of metal bands placed on the vessel (14); by a modification of the Goldblatt renal artery clamp controlled by a flexible cable, the end of which is buried just under the skin (234); by means of a clamp which is closed gradually by a plunger actuated by osmotic pressure (14); and by means of a collar placed around the artery and loosely attached to the thoracic wall (29).

The mortality in acute occlusion of a coronary artery is believed to be reduced in experimental dogs by previous gradual, partial occlusion of the same vessel (29); by ligation of the coronary sinus or great cardiac vein one to four months previously (14, 233); by anesthesia (148); and by prior section of the cardiosensory (and incidentally of the cardiac sympathetic) paths (152). It was sug-

gested (148, 152) that the results of anesthesia and nerve section were due to the prevention of reflex spasm of other coronary arteries, but no valid experimental evidence for this conclusion was presented.

In experimental animals, muscle shortening in the infarcted area is abolished within one to two minutes after coronary occlusion (231). Similar changes have been observed in man by means of the roentgenkymogram (96, 228).⁶ Ligation of the coronary sinus or great cardiac vein did not prevent the immediate failure of shortening in the ischemic area after sudden ligation of a coronary artery (233). A month or more after coronary artery ligation in dogs, shortening was again recorded in the area normally supplied by the ligated vessel whether or not the venous channels had been ligated (233). Although no microscopical changes were observed in the myocardium following a period of ischemia lasting forty-five minutes chemical analysis indicated increases in sodium and chloride ion and no change in potassium ion content; this was interpreted as indicating an augmentation of the extracellular fluid (99).

Narrowing or occlusion of coronary arteries causes regularly the development of intercoronary anastomoses measuring 40 to 200 micra, in comparison to the normal channels of less than 40 micra (21). Following gradual experimental occlusion of the coronary arteries, the re-established circulation is mainly from the first branches of the main rami proximal to the ligation (29). After occlusion of a coronary artery for two to three months, the backflow which could be obtained from the artery distal to the occlusion was increased from a normal value of 1 to 1.5 cc. per min. to 5 to 80 cc. per min., and it had the same oxygen and carbon dioxide content as arterial blood. Successive ligation of the two remaining rami in each case showed that from 7 to 55 per cent of the collateral supply came from each of the remaining main rami. The source of the remainder was not determinable (92). Further attempts to increase collateral supply by production of adhesions between the heart and retrosternal tissue, muscle, or omentum in patients and experimental animals were reported (14, 29, 101).

⁶ Note added to proof: See also, A. M. Master, R. Gubner, S. Dack, and H. S. Jaffe, *The American Heart Journal*, 20, 475 (1940).

While the results seemed to be favorable symptomatically in patients, only a few narrow anastomotic channels were found in three who died after sufficient time had elapsed for collaterals to have developed (14). In experimental animals, sudden ligation of the blood supply to the cardiac grafts had no effect on cardiac function and perfusion of the hearts failed to demonstrate any significant communications between the graft and the cardiac arterial channels (29).

THE PULMONARY CIRCULATION

The pressure ranges in the pulmonary artery of anesthetized dogs in closed chest experiments have been previously studied by reasonably reliable apparatus. For general purposes systolic and diastolic pressures in the pulmonary artery may be placed at about 40 and 10 mm. Hg respectively, giving a pulse pressure of 30 mm. Hg and a mean pressure of about 20 mm. (251).

Katz & Steinitz (124) have recently reported systolic pressures of 25 to 35 mm. and diastolic pressures of 7 to 15 mm. Hg in normal unanesthetized dogs; these approach the lower range of values previously reported. The pressures are not elevated in experimental hypertension. According to Moisset de Espanes (160), ligation of a main pulmonary branch, in dogs with both open and closed chests sometimes proves fatal but more commonly causes only transient fall of arterial pressures. Adams & Escudero (1) found no significant effects from such occlusion of a single branch. The question whether changes in the capacity of the pulmonary bed affect the filling of the left heart and arterial blood pressure during respiratory phases is answered in the affirmative (237). Cahoon *et al.* (33) studied the cause of decreased ventricular output during inspiration through windows in the chest wall. They noticed that the right atrium increased in size during inspiration while the diastolic filling of the ventricles decreased. Reinterpreted, this means that ventricular filling is reduced as atrial pressures increase—a phenomenon which is not likely under normal conditions but was probably caused by their experimental conditions which allowed variable intrathoracic pressures to play upon the atria but not upon the ventricles, thereby creating an atrial stasis during inspiration. Windle (274) reviews the changes in pulmonary circulation at birth.

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PERIPHERAL CIRCULATION

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VENOUS CIRCULATION

Venous pressure.—New methods for measurement of venous pressure by the direct method have been described by Kolff, who has found normal values to range from 30 to 90 mm. water, and by Scaffidi, who places the normal at 91 mm. water. The latter has found no sex difference in venous pressure, but did find a slow decline with age throughout life. The precautions necessary for obtaining reliable results by the direct method have been clearly described by Thomson, Reid & Cohen. The variation in venous pressure with various types of respiratory movements, according to Liedholm, is such as to require their consideration in interpreting changes observed in venous pressure.

The venous pressure during pregnancy is within normal limits, according to Thomson, Reid & Cohen. It is probably only slightly increased in the toxemias of pregnancy. Veal & Hussey report that the pressure in the saphenous vein is increased during surgical manipulation within the abdomen; the pressure in the antecubital vein was not markedly affected.

Nervous control of the venous circulation.—Spinal anesthesia in man causes a slight fall in venous pressure which is associated with a decrease in cardiac output, according to Goldfarb, Provisor & Koster. Smith and his co-workers, on the basis of similar experiments, interpret this as due to mechanical changes resulting from the skeletal muscle paralysis. Y. Henderson long ago emphasized the role of skeletal muscle tonus in the regulation of the venous return to the heart, a view supported more recently by the demonstration by Spychala and by others that evocation of the carotid sinus reflex decreased skeletal muscle tonus. The participation by skeletal muscles in the carotid sinus reflexes has also been inferred by Bacq, Bremer, Brouha & Heymans. However, Schiappoli was unable to produce consistent changes in the human venous pressure by increasing carotid sinus pressure.

Several studies of responses of veins of the extremities have

been reported. According to Abramson & Ferris (1), and Ferris & Abramson (2), pain can cause a venoconstriction in the human forearm without concomitant vasoconstriction. Spontaneous variations in the volume of the hand which are attributed to changing venous tonus have been found by Katzenstein & Abramson. From the observations of Hertzman & Dillon (3) on various areas of the human skin, it also appears that the arterioles and veins of the skin may be independently controlled by the nervous system, the venous responses often lagging behind those in the arterioles.

An interesting demonstration of "voluntary hypercirculation" has been made by Ogden & Shock; two subjects were observed who could accelerate their hearts at command, the acceleration apparently being accompanied by an increased cardiac output, which was attributed to a discharge of blood reservoirs.

Studies of the venous circulation by means of drugs which act on the autonomic nervous system have again demonstrated the venopressor action of epinephrine (Fleisch & Küchler). The rise in venous pressure, according to Scaffidi & D'Agostino, is accompanied either by no change or by an increase in the caliber of the superficial arm veins. These workers also report a rise in venous pressure, together with a decrease in the vein caliber (venoconstriction), resulting from the administration of pilocarpine. Atropine caused a fall in pressure and increased caliber. They conclude that the parasympathetic causes venoconstriction, a view also held by Schiappoli. The data hardly justify this concept, particularly in the absence of anatomically demonstrable parasympathetic fibers from the limbs.

Influence of posture on the venous return.—When a normal man stands, the tendency of gravity to decrease the venous return to the heart is offset by compensatory mechanisms so that no significant fall in cardiac output follows (Goldbloom, Kramer & Lieberman). If he be tilted passively, the compensatory mechanisms may not be fully activated, and fainting may follow. This effect has been the subject of a number of studies.

The compensatory mechanisms include the assistance given to the venous return by postural sway (Hellebrandt, Brogdon & Teppler). Subjects who failed to show the normal progressive rise in intramuscular pressure, when passively tilted, suffered a fall in venous pressure and were likely to faint (Mayerson & Burch). Jacobson has shown that, if a sufficient decline in skeletal muscle

action potentials occurs in a man lying down for fifteen minutes, a fall in arterial pressures will occur on standing.

Vasoconstriction occurring on assumption of the upright posture displaces blood into the venous circulation and so aids in maintenance of cardiac filling. Nielsen, Herrington & Winslow have shown, by studies of skin temperature and heat conductivity, that this postural vasoconstriction is more intense at an environmental temperature of 35.6°C. than in a hotter environment, probably due to competition between the temperature-regulating and the posture-compensating influences acting on the vasoconstrictor center. This compensatory response is weakened by anoxia, according to Gellhorn & Lambert.

The effectiveness of compensation for the upright posture depends upon the blood volume. Scott, Bazett & Mackie have followed the course of adaptation to a warm environment over a period of several days, during which the blood volume rose progressively (Bazett, Sunderman, Doupe & Scott). The rise in diastolic pressure occurring on standing (indicative of vasoconstriction) became less marked as the adaptation to warmth progressed. Conversely changes accompanied adaptation to cold.

Conditions involving marked diversion of blood from its usual channels may impair venous return to such an extent that postural syncope results. Such conditions include exposure to hot climates (Hick, Keeton, Glickman & Montgomery), induction of artificial fever by diathermy (Kopp), administration of sodium nitrite (Stead, Kunkel & Weiss; Kunkel, Stead & Weiss), and indulgence in brief violent exercise (Brogdon & Hellebrandt). The occurrence of a rise in diastolic pressure preceding orthostatic syncope in artificial fever and after violent exercise shows that, in these conditions at least, syncope is not due to a low peripheral resistance.

The influence of posture on the circulation of anesthetized animals has also received attention. Edholm found that chloralosed cats on being tilted (feet down) may show a rise, no change, or a fall in arterial pressure, the last being the most common. When the pressure fell, it was ordinarily followed by a more or less complete recovery. Inactivation of the carotid sinus reflexes did not significantly impair the compensatory responses, but section of the depressor nerves did. Mayerson has made similar studies in dogs. He found that tilting decreased the blood flow in the carotid and fe-

moral arteries, although there was a preliminary increase in the latter. Surprisingly the blood flow in the renal artery was never decreased as much as in the other arteries studied.

Influence of various factors on the venous return.—Mechanical pressure applied to the body of animals by immersion in water at the indifferent temperature increases the blood flow in the carotid artery in proportion to the fraction of the body immersed, according to Heite & Lerche.

Only when the intrapericardial pressure of a patient with therapeutic pneumopericardium was raised above 145 mm. water, did the venous pressure and circulation time increase. At 265 mm. water, signs of circulatory failure appeared. The authors, Adcock & Barnwell, concluded that the venous pressure must exceed the intrapericardial pressure by at least 35 mm. water to maintain the circulation.

The fall in intrathoracic pressure caused by deep inspiration evokes a decrease in arm volume; but, on account of contraction of the diaphragm and the consequent increase of intra-abdominal pressure, an increase in leg volume also results, according to Théodoresco & Aslan. Forced expiration with closed glottis (Valsalva procedure) increases both arm and leg volume and greatly decreases the filling of the heart. This effect has also been studied in detail by Liedholm, who found a wide variety in the venous pressure changes produced. An attempt to use it as a test for heart efficiency failed.

Intestinal distention has been found to impede venous return from the legs; shock with extravascular loss of fluid from the blood may follow (Bellis & Wangenstein).

Trapping blood in three extremities by means of cuffs inflated to diastolic pressure for about ten minutes (followed by a pressure of 250 mm. Hg) may cause sufficient reduction in venous return to the heart to cause collapse. Estimates of blood volume before and after this procedure indicated that about 720 cc. of blood had been diverted from the head, trunk, and arm. Its use in place of venesection for rapid relief of venous congestion in acute cardiac failure is supported by these observations.

The venous circulation in heart disease.—In the second edition of his book, *Failure of the Circulation*, T. R. Harrison again emphasizes the predominant role of high venous pressure in the production of the signs and symptoms of cardiac failure.

The mechanism whereby the venous pressure is raised in cardiac failure has been studied by several workers. Starr & Rawson found that, in a mechanical model of the circulation with pumps obeying Starling's law of the heart, the arterial and venous pressures characteristic of clinical congestive failure cannot be produced by weakening of the heart alone. To increase the "static blood pressure" (the pressure in the circulation when the heart is stopped), mechanisms such as general vasoconstriction, pressure on the vessels from without, and increase in blood volume must be introduced. Measurements of static blood pressure made on patients shortly after death gave an average of 7.6 cm. water in the absence of heart disease, an average of 20.3 cm. in the presence of congestive failure. Alteration in cardiac function is believed inadequate to explain the increase in venous pressure seen with congestive failure. A similar conclusion has been reached by Condorelli. Cossio & Berconsky claim that the increased venous pressure accompanying mitral stenosis results in part from changes in the position of the heart and aorta which mechanically compress the superior vena cava.

Variation in the venous pressure changes produced by a standardized exercise test are reported by Van Nieuwenhuizen. Although in severe degrees of congestive failure he made the classical finding of a high resting venous pressure with long-lasting increase after exercise, cases were observed in which exercise caused a fall below the resting level. This occurred in patients with early decompensation in whom the dyspnea of effort was marked. In some of them the resting venous pressure was not elevated.

ARTERIAL CIRCULATION

Blood flow in arteries.—The time course of blood flow in the aorta of the dog during the cardiac cycle has been studied by Green, Schroeder & Paschold. The flow accelerates abruptly with the systolic rise in pressure, reaches its maximum at the first pressure peak, declines during mid-systole, and drops sharply to zero as protodiastole begins. Backflow then occurs, equalling at the incisura about one third of the maximum systolic forward flow. The forward flow resumes, accelerates until mid-diastole, then decreases progressively to the end of diastole. Effects of increased venous pressure, epinephrine, asphyxia, pitressin, and sodium nitrite are described.

Backflow also occurs in the arteries of the foot, as has been revealed in the plethysmographic study of Wright & Phelps. Since it is reduced by vasodilatation, it is attributed to the fact that the pulse wave carries forward more blood than can immediately pass through the constricted arterioles. Failure to consider this backflow may invalidate estimates of blood flow made by means of volume pulse recorders (oscillometers).

Physical properties of arteries and their relation to arterial pressure and flow.—Lahy, employing a piezographic sphygmomanometer, found that after arterial occlusion the arterial pressure falls in an exponential curve which approaches asymptotically the minimum pressure which will maintain the circulation. By the use of a formula developed for the purpose, this minimum pressure was estimated to be between 5.7 and 8.2 mm. Hg. Dow & Hamilton occluded the aorta of dogs and studied the curve of declining pressure measured peripherally to the point of occlusion. They found the pressure to fall to a minimum of about 20 mm. Hg, at which the blood flow stopped. This they attribute to the anomalous apparent viscosity of blood which increases with slowing of the circulation. The relation of the rate of fall of pressure to the pressure existent at a given moment varies in different segments of the aorta. Accordingly the authors felt that all attempts to formulate these relations mathematically have been unsatisfactory. The curves obtained confirmed previous findings that the volume-elasticity coefficient of the arterial reservoir increases with rising pressure.

Physical properties of arteries and their relation to the pulse wave.—According to Hume, the resistance to a stretching force (measured by Young's modulus) of rings taken from the aorta of rats decreases with advancing age, and with rising temperature; it is slightly increased if the rats have been subjected to toxic doses of lead or alcohol.

Hürthle has recorded simultaneously the changes in pressure and volume in excised segments of arteries perfused with a pulsatile stream of Ringer's solution. He reports that epinephrine caused an increase in the ratio of volume to pressure (a relaxation), while parathyroid extract and, less regularly, posterior pituitary extracts decreased the ratio. The concentrations of hormones used seem so great as to deprive the observations of significance in relation to physiological processes.

Wezler & Knebel used the Valsalva experiment to evoke arte-

rial changes in intact human subjects. They found that the modulus of elasticity and the period of vibration of the aorta and radial artery are altered reflexly by this procedure, the reflexes arising in pulmonary receptors and in the carotid sinuses and acting on the muscular coats of the arteries.

The pulse wave velocity in the hand arteries is normally about 20 per cent less than in the radial artery. Epinephrine in physiological doses increases the velocity in both regions, the elastic properties of the vessels being altered by contraction of the smooth muscle, according to Greven & Federschmidt. Constriction of the radial artery was found by Hertzman & Dillon (2) to accompany the general arteriolar constriction on immersion of a hand in cold water, but not that evoked by psychic stimuli, loud noises, or deep inspiration.

Matthes, Gross & Göpfert (1), recording the volume pulse of various skin areas simultaneously with the pulse wave in a larger artery, have concluded that the portions of the arterial tree just central to the region of maximum peripheral resistance have elastic properties whereby they serve to promote continuous forward flow of blood in a manner homologous to the elastic function of the aorta. A similar study of the pulse wave form and velocity in extreme bradycardia and in hypertension has been made by the same investigators (2).

Hamilton (2) has described in the pressure pulse in some arteries (such as the brachial) certain standing waves which arise by reflection from the arterioles and are accordingly abolished by intra-arterial injection of vasodilator drugs. The development of the characteristics of the pulse of aortic stenosis has been studied by Dow. Dillon & Hertzman have commonly found among hyperreactors to the cold pressor test certain alterations in the shape of the radial and digital pulses and in the time relations between them.

Two new techniques for recording the pulse have been described; the one by Lahy employs a piezographic principle; the other, by Fabre, depends upon the change in resistance to a high frequency current flowing between two electrodes placed on the skin along the course of an artery, the resistance being altered by the change in volume of the artery as the pulse wave traverses it.

Estimation of the stroke volume of the heart from arterial pressure and pulse data.—Deppe & Wetterer, and Wetterer & Deppe have determined the stroke volume of cats and rabbits by means of an

electromagnetic flow recorder, and have compared it with values calculated by various equations for its prediction from the systolic and diastolic pressures, and the pulse wave velocity, which were also measured. The equation of Broemser & Ranke gave the best agreement; under resting conditions the average discrepancy was 15 per cent, the maximum, 34 per cent. During circulatory responses to asphyxia and certain drugs, the error may amount to 90 per cent. The authors warn against the application of these conclusions to larger animals.

Arterial pressure: methods.—Waters has applied the Friedman oscillometer to the measurement of the arterial pressure of dogs by an indirect method not requiring a carotid cuff. Rodbard has found that the arterial pressure of properly quieted rabbits can be obtained without anesthesia by direct puncture of the femoral artery. Moers & Schlienzy have devised a method of measuring the pressure in the central artery of the rabbit's ear by occlusion. Beyne & Gougerot have described a method whereby the excursions of a mercury manometer can be transmitted electrically and recorded at a distance.

The arterial pressure of unanesthetized dogs has been determined in one leg by the auscultatory method and simultaneously in the other by Hamilton's method involving direct arterial puncture. Cady & Herrick found that the systolic pressure obtained directly might be as much as 40 mm. Hg higher than that obtained by auscultation; the diastolic pressure obtained directly agreed with that obtained by auscultation more satisfactorily when complete disappearance of sounds, rather than the change of quality, was used as the criterion of diastolic pressure. Some observations on the origin of the Korotkoff sounds have been reported by Erlanger.

Standardization of methods of determination of arterial pressure in man.—The American Heart Association and the Cardiac Society of Great Britain and Ireland have made a joint recommendation for the standardization of arterial pressure readings, some features of which will be mentioned here. The sitting posture is preferred by the American committee, the recumbent posture by the British. A rest period of ten to fifteen minutes after the last exercise is recommended. The criterion of systolic pressure should be either the first sound heard regularly as the cuff pressure falls or the first return of the radial pulse, the higher level being recorded. The diastolic pressure is to be read at the point where the auscultatory sounds suddenly become dull or muffled. The American committee

recommends that, if there is a difference between that point and the level at which the sounds completely disappear, both values should be noted, as, for example, 140/80-70. It is hoped that these recommendations will be adopted by practicing physicians, medical teachers, insurance examiners, and all others concerned.

Arterial pressure: regional differences.—In the right arm of normal young adult males the systolic arterial pressure averaged 1.6 mm. Hg. higher (standard error, 0.36) than in the left arm while the diastolic pressure was 0.36 mm. higher (standard error, 0.18). The difference was statistically significant in over 70 per cent of subjects, according to Shock & Ogden. A similar preponderance of higher pressures in the right arm of medical students was found by Nice, Ehlers & Warner.

The systolic pressure in the femoral artery, which is normally higher than that in the carotid artery, is brought down to the latter value when the standing waves in the femoral pressure pulse are eliminated, according to Hamilton (2).

Early in hemiplegia the arterial pressure on the paralyzed side may be considerably higher than on the normal side. The difference is attributed by Hauss to arteriolar constriction on the affected side, rather than to difference in the state of the skeletal muscles.

Arterial pressure: normal and pathological values.—An important statistical analysis of the arterial pressures of a large group (7,478 men and 3,405 women) has been reported by Robinson & Brucer. The mean values (with standard errors of the means) were the following: for men, 121 ± 17 mm. Hg systolic, and 74 ± 11 diastolic; for women, 117 ± 11 systolic, and 71 ± 12 diastolic. After the age of thirty years, the modes of five-year age groups were consistently below the means, on account of the skewing effect of the presence of subjects with hypertension. After exclusion of subjects with systolic pressure over 140 mm. Hg and diastolic pressure over 90 mm. Hg as hypertensive, the remaining "normal" subjects have the following pressures: men, 115 ± 10 mm. Hg systolic, and 72 ± 8 diastolic; women, 112 ± 10 , systolic, and 70 ± 8 , diastolic. Among the normal male subjects, the arterial pressure levels did not increase with age; among the female subjects the mean values, which were lower in early adult life than those of males of the same age, rose progressively until at about forty years they reached the male level. The authors also studied the determinations made repeatedly over periods of five to ten years on the same subjects. The pressures

which were originally low and stable did not rise; however, those which were originally either high or unstable increased, commonly to the point of frank hypertension. They estimate that slightly more than 40 per cent of the adult population is actually or incipiently hypertensive. (This subject is considered further in the section on the cold pressor test.)

The relation of body weight to the arterial pressure has interested several groups of workers. Short & Johnson have reported that in their 2,858 overweight and 658 normal subjects, when classified by weight, the incidence of diastolic hypertension rose from about 4 per cent in those not departing more than 5 per cent from their ideal weight, to 32 per cent in those who were 41 per cent or more over their ideal weight. The relation persists when corrected for age. The maximum difference was in the age group of 50 to 59 years, wherein the average systolic pressure difference between normal and overweight persons was 11 mm. Hg, and the diastolic 4 mm. Hg. According to Robinson, hypertension is associated with the lateral or broad body type, which is prone to become obese, rather than with obesity as such. In the linear or slender body type there is a positive correlation between obesity and hypertension; in the lateral type none has been found. The authors conclude that the role of obesity is small. However, body weight is itself a factor, for Wood & Cash, who controlled the weight of normal and hypertensive dogs by dietary means, found that systolic pressures rose with weight gain and fell with weight loss; the diastolic pressures varied but slightly.

VASOMOTOR MECHANISMS: CENTRAL AND PERIPHERAL

Reviews.—General reviews of the vasomotor nerves, centers, and reflexes have been written by Fog and by Nonidez. The structure and responses of the arteriovenous anastomoses have been reviewed by Clark.

Plethysmographic methods.—New plethysmographs for measuring blood flow by the Hewlett-van Zwaluwenburg principle have been described by Ferris & Abramson, by Wright & Phelps, and by Burton. Kolin has recorded vasomotor responses by determining the apparent weight changes occurring in a liquid when a limb immersed in it (but suspended from outside) undergoes a volume change.

Criteria of vasomotor reactions.—In an attempt to evaluate arterial pressure changes as quantitative criteria of vasomotor

reactions, Dingle, Kent, Williams & Wiggers measured the cardiac output (by means of a cardiometer) and mean arterial pressure of dogs under barbiturate anesthesia. From these data and Poiseuille's law, they calculated the peripheral resistance. In the pressor responses to central vagus stimulation, aortic occlusion, and injection of epinephrine and pitressin, the absolute rise in arterial pressure, its relative increase, and the calculated peripheral resistance change—regarded as criteria of the vasoconstriction—often give conflicting estimates of the relative magnitude of the various responses. This conflict arose partially because changes in cardiac output were almost invariably present, and partially because, according to the authors, the relationship between systolic discharge and aortic capacity affects the mean pressure.

The interpretation of plethysmographic records as criteria of vasomotor reactions has received critical evaluation. It is agreed by Holt & Lawson, by Abramson & Ferris (1), and by Hertzman & Dillon (2) that the volume changes recorded are due in larger part to changes in the blood content of the capillaries and veins than to changes in the volume of the arterioles. This is particularly true in the forearm, according to Abramson & Ferris, where blood flow rate and volume may change in opposite directions. Moreover, when volume changes in the hind leg of the dog are prevented by application of a cast or tight bandage, Holt & Lawson found that procedures which alter the peripheral resistance (such as intra-arterial injection of epinephrine or nitroglycerine, or section of the sciatic nerve) exert the same effect on blood flow as when the volume of the limb is permitted to change. Hertzman & Dillon (2) recorded simultaneously finger volumes and finger volume pulses. They believe that the volume flow can be estimated by the product of the volume pulse and the heart rate. The amplitude of the volume pulse is inversely proportional to the arterial tone. Accordingly, when the increased volume is accompanied by an increased volume pulse, arterial dilatation may be concluded to have increased the volume. Nyssen & Busschaert caution against mistaking the plethysmographic record changes produced by involuntary muscular movements or tonus changes for those due to alteration in blood vessel volume.

Vasoconstrictor centers and paths.—The role of the hypothalamus in cardiovascular regulation has been investigated by Pitts, Larrabee & Bronk. These workers found that the spontaneous activity of sympathetic nerve trunks, and the changes produced

therein by reflexes, are not altered by extirpation of the hypothalamus. Stimulation of the hypothalamus at rates above ten per second increases, but at lower rates decreases the activity of these nerves. It appears that, while the hypothalamus is not essential to vasoconstrictor tonus, it can alter this activity in relation to function mediated by this part of the brain.

A participation of the paleocerebellum in vasomotor regulation, whereby it integrates vascular with muscular responses in bodily activity, has been claimed by Moruzzi. Electrical stimulation of the vermis of the anterior lobe of the decerebrate cat inhibits the pressor effect of concurrent stimulation of the sensory nerves and the depressor effect of stimulation of the central end of the vagus or evocation of the carotid sinus reflexes. Since the effects are still demonstrable after curarization, they are not secondary to changes in skeletal muscle tonus.

Monnier has found that in the bulb of the cat (explored by means of the Horsley-Clarke apparatus) stimulation of the lateral reticular-formation yielded increase in the arterial pressure, while stimulation of the dorsomedial structures, the calamus scriptorius, obex, and fasciculus solitarius gave decreases. The responses were not affected by curarization.

The existence of a tonically active vasoconstrictor mechanism in the spinal cord, connecting with neurons reaching the periphery by nerves outside the sympathetic nervous system, is claimed by Brown & Maycock. Section of the spinal cord of sympathectomized decerebrate cats caused a fall of arterial pressure even after muscular paralysis with curarine.

The efferent paths of vasoconstrictor impulses in the spinal cord of man have been located in the anterior and anterolateral columns. According to Hyndman, Wolkin & Paul, bilateral section of these parts, preferably at the eighth cervical segment, has reduced the arterial pressure of patients with essential hypertension to a normal level for four to six months.

Certain other studies of the central vasomotor mechanisms are discussed by Sheehan in the chapter on the autonomic nervous system in this volume.

Tonic activity of the vasoconstrictor center.—Smith and his co-workers have issued a sharp challenge to the currently accepted view that the splanchnic arterioles are continually maintained in a state of partial constriction by tonic activity of the vasoconstrictor mechanisms. They review the evidence on which this concept is

based and pertinently point out that much of it was obtained on animals under anesthesia, a condition which of itself almost regularly originates or increases vasoconstrictor activity. Whether tonic vasoconstriction is also present in the resting unanesthetized human being cannot be settled by such experiments. The induction of spinal anesthesia in normal men in the supine position was not invariably followed by a fall in arterial pressure. When it did fall, the systolic pressure decreased more than did the diastolic, the latter rarely falling below 60 mm. Hg. The investigators attributed the fall in arterial pressure to decreased cardiac output (caused by relaxation of the skeletal muscles of the legs, abdominal wall, and some of those involved in respiration), rather than to a reduction in pre-existent vasoconstrictor tone in the anesthetized regions. Their emphasis on the role of reduced venous return seems justified, for Goldfarb, Provisor & Koster have shown in similar studies on man during spinal anesthesia that the venous pressure and cardiac output are reduced. A second factor tending to reduce arterial pressure, a vasodilatation in the skin of the lower extremities (demonstrating a tonic vasoconstriction in this vascular area), was present. However, no observations were made whereby it might be determined whether the fall in cardiac output and cutaneous peripheral resistance were sufficient quantitatively to account for the observed level of arterial pressure. The inference that no additional splanchnic vasodilatation occurred seems to the reviewer to be deduced from evidence too indirect to be convincing. It should be admitted that the absence of an increase in kidney blood flow (measured by diodrast clearance) favors Smith's view, in showing that, as far as the renal circulation is concerned, there is no relaxation of vasoconstrictor tone. Even if further study should show that the splanchnic peripheral resistance is not reduced in spinal anesthesia, the possibility would still exist that compensatory mechanisms, possibly hormonal, might be brought into action quickly and so maintain the tonic contraction of the arteriolar muscles previously maintained by the vasoconstrictor nerves. However, it appears to the reviewer that proponents of the view that there is a neurogenic vasoconstrictor tone in the splanchnic area are, as a result of the work of Smith and his associates, definitely on the defensive. It should be emphasized that the conclusions of these workers apply only to man in the supine position and under basal conditions.

Spontaneous waves in plethysmographic records from various

skin areas have been observed by Hertzman & Dillon (1) and by Burton (1). The former, impressed by the independence of the vasomotor behavior in different areas, are inclined to regard them as examples of the activity of selective vasomotor patterns in the skin. Burton noted that constriction, occurring simultaneously in fingers and toes, was accompanied by cardiac acceleration and a rise in arterial pressure. Burton & Taylor regarded the absence of participation of other areas in the constriction seen in the fingers and toes as evidence rather of their relative vasomotor inactivity than of independence. They believe that these "spontaneous" waves are evidence of mass activity of the vasoconstrictor center. Since the waves were accelerated in a cold environment, the authors suggested that "the vasomotor center may have an intrinsic property of responding to a constant intensity of afferent stimulation by an intermittent rhythmic discharge, the rhythm of which is modified by the intensity of stimulation."

An extensive review of the tonus of blood vessels after severance of their nervous connections with the central nervous system has been written by Hermann.

Intracranial pressure and the vasomotor centers.—When the intracranial pressure is raised above the level of the arterial pressure, the latter increases sharply. This, the Cushing reaction, has been studied by Yesinick & Gellhorn. Like the response to asphyxia and unlike that to anoxia, it persists after removal of the buffer nerves; it is therefore due to asphyxia, not anoxia, of the medullary centers. It is increased by either anoxia or hypoglycemia.

Scott has studied the physiology of concussion in the dog. A blow on the head, sufficiently powerful to raise the intracranial pressure considerably above the arterial pressure, causes loss of consciousness even if the pressure be maintained for only a second. A brief but complete cerebral anemia is apparently adequate. A series of such periods of anemia is suggested as the cause of the syndrome of "punch drunk."

Peripheral factors affecting vasoconstriction.—Late in adrenal insufficiency, stimulation of the splanchnic nerve or injection of nicotine, pitressin, or barium chloride yields a subnormal increase in arterial pressure, according to Armstrong, Cleghorn, Fowler & McVicar. Cleghorn has added the fact that the pressor response to carotid occlusion is reduced. Since the pressor response to epinephrine injection is normal, the adrenal insufficiency is considered

to interfere with the production of the sympathetic humoral transmitter at the vasoconstrictor endings. This view is supported by the fact that ephedrine, which protects the transmitter from destruction, also gives a subnormal pressor response. Fowler, Clarke & Cleghorn have shown that the decrease in the pressor response to pitressin appears quite early in adrenal insufficiency. According to von Marsovszky, who confirmed this finding, the rise in arterial pressure due to epinephrine and other pressor drugs was not reduced at this time, a fact demonstrating that the impaired response to pitressin was not due to a poor reactivity of the vascular musculature.

An apparent reversal of the vasoconstrictor response of the vessels of the cat's paw to epinephrine, dependent on the state of the temperature-regulating mechanism, has been reported by Goetz (1). In cooled animals constriction of the skin vessels is suppressed and dilatation takes place. This dilatation may be an expression of epinephrine's central action of depressing the defence against overcooling, which was postulated by Hall & Goldstone on the evidence that epinephrine inhibited shivering in anesthetized cats.

Vasodilator nerves.—The question as to whether the dorsal root vasodilators can be activated reflexly has been reinvestigated by Dole & Morison. Since there is no difference in the reaction of the totally denervated paw and the sympathectomized paw on stimulation of the depressor nerve or the nerve of Hering, dorsal root vasodilators play no part in the pattern of these reflexes.

The view that curare paralyzes the antidromic vasodilatation has been shown to be incorrect by Brown & Maycock, at least in the cat.

VASOMOTOR REACTIONS

In addition to the studies of vasomotor phenomena considered in this section, many are described elsewhere in this volume: those involved in temperature regulation by Bazett; those in the hypertension of renal ischemia by Leiter; those in exercise by Steinhaus and a number involved in viscerosomatic relations by Sheehan.

The cold pressor reaction and vasomotor reactivity.—The rise in arterial pressure evoked by immersion of a hand in ice water is being used as a standard test for vasomotor reactivity. Miller & Bruger classified as hyperreactors those subjects showing a systolic

pressure rise greater than 22 mm. Hg. These comprised 39 per cent of their normal subjects. Subjects with hypertension commonly show a greater rise in pressure than occurs in normal persons, according to Hines (1) and Thacker. Hines believes that hyperreactivity may be an inherited characteristic and that it represents an antecedent to, or a latent phase of, essential hypertension.

Since the circumstances surrounding the first visit of a patient to a clinic constitute a psychic pressor stimulus equivalent to some extent to the cold pressor stimulus, Hines (2) correlated the arterial pressure of patients, measured on the occasion of their first visits to the Mayo Clinic, with the subsequent development of hypertension as determined on the return of these patients ten or twenty years later. The incidence of hypertension in these groups was less than 4 per cent in those with an original systolic pressure below 140 and a diastolic below 85 mm. Hg. In the patients in whom both pressures exceeded the above values, the incidence of hypertension was over 80 per cent. Hines considered that his results support the view that excessive variability or excessive responses of the usually normal arterial pressure to stimulation should be considered as evidence of a possibly prehypertensive state.

Barostatic reflexes (carotid sinus and aortic depressor).—Addison has studied the histology of the mammalian carotid sinus. Two papers have appeared on the mechanism of stimulation of the carotid sinus. Sweeney has been unable to confirm claims of earlier workers that the carotid sinuses exerted a reflex pressor effect when the arterial pressure level was unusually low. The sensitivity of the pressoreceptors is in some degree dependent on the respiratory gases in the blood, oxygen increasing and carbon dioxide decreasing it, according to Bielinski & Wierzuchowski. Van Harreveld & McRary have reported that hypoglycemia may reduce the effectiveness of the depressor reflex, while subsequent administration of glucose increases the magnitude of the arterial pressure fall.

The hypertension produced by bilateral section of the carotid sinus and depressor nerves has usually been transient. Nowak has now produced in dogs a hypertension lasting at least three years. The operation involved, in addition to section of the four moderator nerves, excision of the bifurcations of the carotid arteries. Remissions in the resultant hypertension were attributable to inanition. The participation of a humoral factor in this form of hypertension is claimed by Hermann, Jourdan & Debrieu, since the

blood of the dogs acquires vasoconstrictor properties (as tested by perfusion of frog legs) after section of the four moderator nerves. Since the arterial pressure of such dogs is caused to fall by the administration of piperidomethyl-3-benzodioxane (F933), the secretion of epinephrine is believed to be increased. However, after removal of one adrenal and demedullation of the other, Hermann, Jourdan & Debrieu found the blood still to contain a vasoconstrictor substance, assumed to be sympathin.

Grimson has shown that in sympathectomized dogs subsequent section of the moderator nerves increases the arterial pressure above the level which existed before the second operation, so demonstrating that these reflexes can exert a depressor effect by mechanisms outside the sympathetic nervous system. Bacq, Bremer, Brouha & Heymans have also studied vasomotor reactions in sympathectomized animals. Although in dogs no such responses are demonstrable, in cats (decerebrated and vagotomized) carotid occlusion increases and sinus nerve stimulation decreases the arterial pressure, provided that the animals are in good health. The authors attribute the persistence of the responses not to activity of dorsal root vasodilators, but to changes in skeletal muscle tonus and movements, including respiration. (See also the section on nervous control of venous circulation.) The predominance of depressor responses in decerebrated sympathectomized cats (with carotid sinuses inactivated) is attributed by Brown & Maycock to vasodilatation in muscles which contracted reflexly in response to the stimuli used. The depressor responses were almost completely abolished by curarine.

The response to carotid sinus reflex evocation includes vasodilatation in the blood vessels of the skeletal muscles (Grimson & Shen), but not constriction of the spleen (Gregersen). Extravascular effects include decreased oxygen consumption and carbon dioxide output (Heymans & Delaunois), even when artificial respiration is used to avoid the complication resulting from the reflex apnea produced. It is interesting to speculate whether this may be due to the decrease in skeletal muscle tonus, discussed previously in the section on nervous control of the venous circulation.

In experimental renal hypertension, the barostatic reflexes play the same part in the regulation of arterial pressure that they do in normal animals, according to Goldblatt *et al.* Dock believes that in

renal hypertension the "set" of the vasomotor center is altered so as to regulate the arterial pressure at a new high level, the barostatic reflexes behaving in such a manner as to restore the pressure, when altered, to this new high level.

Rovenstine & Cullen claim that digitalis, morphine, avertin, and light ether anesthesia may increase the activity of the carotid sinus reflex and so predispose to collapse in operative attack on this region. According to Hauss & Shen (2), ephedrine reduces both the depressor and pressor effects of carotid sinus origin.

Atrial pressor reflex.—Waele & Van deVelde (2) have described a pressor reflex which is evoked by a fall in intracardiac pressure and the afferent path of which lies in the first, second, and third thoracic dorsal roots. They regard it as a protective mechanism tending to counteract the effect of a decreased venous return to the heart. Goormaghtigh & Pannier have described the structure of the supracardiac artery as being similar to that of the carotid sinus.

Chemoceptor reflexes.—In this and the following section only papers not considered in the comprehensive review of Schmidt & Comroe will be discussed. Hollinshead has reviewed the anatomy and embryology of paraganglia.

Goormaghtigh has described the glomus caroticum of the cat as consisting of a system of arteriovenous anastomoses. The arterial portion has sympathetically innervated smooth muscle in its walls, while the venous portion has chromaffin paraganglionic cells surrounded by sensory endings. The paraganglionic cells are identified as the chemoceptors. Goormaghtigh & Pannier have also described paraganglia in the region of the heart. These may be receptors for the pressor response observed by de Waele & Van deVelde on injection of acid buffer solutions or sodium sulfite into an atrial vein. The authors consider this to be a chemoceptor reflex homologous with those arising in the carotid and aortic bodies. Its afferent path is similar to that of the atrial pressor reflex.

According to Verdonk, the responses of the monkey's chemoceptors to intracarotid injection of sodium sulfide, potassium cyanide, lobeline, nicotine, and acetylcholine are similar to those of the lower mammals. Contrary to the earlier report of von Euler, Hauss & Shen (1) claim that potassium chloride in threshold doses acts on the chemoceptors so as to produce a fall in arterial pressure, a finding which may be of interest in relation to the role of potassium in excitation processes.

Vasomotor responses to anoxia, hypercapnia, and hypocapnia.—According to Van Harreveld & McRary, the pressor response to anoxia is favored by a certain degree of depression of the barostatic reflexes, such as may result from moderately deep narcosis or hypoglycemia.

Asphyxia causes vasoconstriction in the skin of the dog, according to Malméjac & Desauti, which is in part due to circulating epinephrine, or, after adrenalectomy, to sympathin. Inhalation of 6.4 per cent carbon dioxide by normal men causes decreased blood flow in the hands; after sympathectomy it causes an increase attributed to local vasodilatation. (This response is suggested as a test for the presence or absence of sympathetic fibers.) Apparently hypercapnia in man does not cause epinephrine secretion in quantity adequate to overcome local vasodilatation. Gellhorn, Kiely & Hamilton have reported that hypoglycemia increases the pressor response to carbon dioxide inhalation. Since this is also true after removal of the moderator nerves, carbon dioxide acts on the vasomotor center directly.

Hypocapnia, even if severe, when produced by overventilation causes only a temporary fall in arterial pressure, according to Seevers, Stormont & Hathaway. The pressure soon rises to or above the original level during the continuance of the overventilation. In the absence of general anesthesia, no fall occurs, compensatory reactions (which are depressed in general anesthesia) being adequate to overcome the loss of the pressor effect of carbon dioxide. These results speak against the acapnia theory of shock.

Possible liberation of a pressor hormone from the posterior lobe of the pituitary.—Sattler has confirmed earlier reports by Chang *et al.* that stimulation of the central end of the vagus in dogs, in which the only connection between the head and trunk was vascular, caused an increase in arterial pressure, which was abolished by hypophysectomy and was attributed to a release of a pressor hormone from the posterior lobe. Since this pressor response was absent in dogs in which the supraopticohypophyseal tract had been cut some time previously, its disappearance in acute experiments after hypophysectomy was not due to the trauma and shock of the operation. Lombroso & Martini also believe that central vagus stimulation releases a posterior pituitary hormone.

Clark & Wang demonstrated a pressor effect on hypothalamic stimulation in vagotomized cats in which the cervical spinal cord

had been cut some time previously. The latent period was over fifteen seconds and the rise persisted well over one minute. The authors attributed the pressure rise to the release of a pressor hormone, probably from the posterior pituitary. Sattler found, in his animals with crushed spinal cords, that stimulation of the infundibulum caused a rise in arterial pressure.

It seems fairly clear that a pressor hormone can be secreted by the posterior lobe under certain experimental conditions. As yet, however, there is no evidence that such a process plays a role in vasomotor reactions.

Vasomotor reactions of hormones (other than epinephrine and pitressin).—Estrogenic hormones given to the human male cause an increase in finger volume without increasing the rate of blood flow. The capillaries and venules are dilated, but not the arterioles, according to Reynolds & Foster (1). They have made similar observations (2) on the skin and nasal mucosa of women, and on the skin or the ear of the ovariectomized rabbit. These effects are present after denervation. Ratschow & Steckner have claimed that hypophysectomy prevents the vasodilator action of sex hormones; they attribute the vasodilatation to liberation of acetylcholine.

Thyroxin has, according to Brull, a peripheral vasodilator effect on the blood vessels of the kidney. Insulin, in the massive doses used in the treatment of schizophrenia, causes an increase in blood flow in the hand, arm, and leg at the height of the hypoglycemic response. This is attributed to the increased cardiac output by Abramson, Schkloven, Margolis & Mirsky.

Uterine ischemia.—Ischemia of the gravid uterus of several species of animals, produced by clamping the aorta below the renal arteries, has been shown by Ogden, Hildebrand & Page to cause a slow moderate rise in arterial pressure. Clamping the aorta at this level in nonpregnant animals, or after removal of the uterus, fails to increase the arterial pressure. The authors believe that circulatory insufficiency to the gravid uterus may be responsible for the hypertension sometimes occurring in abnormal pregnancy.

CIRCULATION IN SPECIAL REGIONS

Spleen.—Mackenzie, Whipple & Wintersteiner have studied the blood flow in the spleen microscopically by means of transillumination. In the relaxed spleen, the flow in the pulp spaces is intermittent due to momentary obstruction by columns of cells;

when the capsule, trabeculae, and arterioles contract (in response to epinephrine, anoxemia, hemorrhage, etc.), the pulp is compressed and the flow proceeds as if in a functionally closed circuit. The capsule contracts rhythmically even in the absence of specific stimulation.

Grindlay, Herrick & Mann have applied thermostromuhr methods to the measurement of blood flow in the splenic artery and vein of the unanesthetized dog. Stimuli which cause the spleen to constrict effect at least a transient increase in the flow in the vein. The flow rate in the artery (and after the initial period in the vein) may be either decreased, as with hemorrhage, or increased, as after transfusion or in shivering, digestion, and exercise. In general, the flow through the spleen is controlled in the same manner as that through the other splanchnic viscera. Grindlay, Herrick & Baldes, using the same technique, studied the rhythmic waves of blood flow through the spleen.

Gastrointestinal tract.—Reflexes arising from heating or cooling the skin have been shown by Kuntz & Haselwood to produce vasodilatation and vasoconstriction in the regions of the gastrointestinal tract innervated from the same spinal segments.

Goetz (2) has found that epinephrine yields predominantly dilator effects on the blood vessels of the intestines. Its action here related to blood distribution rather than to the increase in arterial pressure.

Lawson & Chumley found that the blood flow through the intestine is not reduced by distention of the lumen until the intraluminal pressure exceeds 30 mm. Hg. Following release, an increased pressure occurs, attributable to reactive hyperemia.

Portal and hepatic circulation.—Enesco & Busila have measured the hepatic circulation time in dogs by determining how soon after injection into the portal vein potassium ferrocyanide can be detected in the inferior cava. The time varied from 6 to 10 sec., but was increased to from 12 to 23 sec. by ligation of the hepatic artery. A forwarding effect of hepatic artery flow on portal flow is also suggested by the finding of Patrassi & Baggio that ligation of the artery leads to venous congestion of the spleen. Zopff has studied the mechanical and nervous factors involved in the depot function of the liver and emphasized its failure as a factor in various forms of collapse.

Grodins, Osborne & Ivy have shown that certain pure bile salt

preparations increase hepatic artery flow when they stimulate bile secretion.

Katz & Rodbard have analyzed in dogs the interaction of vasomotor responses of the various component parts of the hepatic circulation to the administration of a number of drugs. Epinephrine causes a decrease in portal flow, followed by an increase. In the first phase the liver vessels constrict while the hepatic vein sphincters relax, so forcing the hepatic depot blood into the general circulation. Histamine traps blood in the liver by activating the hepatic vein constrictors, while nitrites cause a pooling of blood in the preportal area.

Kidney.—The physiology of the renal circulation, considered by Leiter in this volume, has also been reviewed by Smith. The circulatory phenomena involved in the hypertension produced by renal ischemia have been reviewed by Blalock.

Skeletal muscle.—The general review on muscle circulation by Malméjac may be recommended. The influence of muscle contraction on the blood flow therein has been studied. Van Dijk, using direct microscopic observation of the blood flow in the popliteal artery of the frog, found that isometric contraction of the gastrocnemius muscle stimulated directly caused a complete cessation of flow; if the tetanus lasted long enough, the flow finally resumed. The mechanical obstruction was believed to have occurred in the intramuscular arteries rather than in the capillaries. Barcroft & Millen followed the blood flow in the human gastrocnemius muscle in sustained voluntary contraction, employing a thermoelectric measurement of muscle temperature. Contraction strengths from 0.05 to 0.1 of the maximal value are accompanied by hyperemia; however, contractions between 0.2 and 0.3 of the maximal strength are not accompanied by increased flow (probably due to mechanical occlusion). A marked hyperemia follows relaxation. The results cited above are in conflict with many of those derived from studies on anesthetized animals, probably because in the latter the contractions were too weak to interfere with the blood flow.

Although Grimson & Shen have shown that the vessels of skeletal muscles of dogs participate in the vasoconstriction produced by direct sympathetic stimulation, by epinephrine injection, and by carotid sinus reflex withdrawal, Friedlander, Silbert & Bierman claim that in human calf muscles the blood flow is decreased by spinal anesthesia, by immersion of hands in hot water,

and by typhoid vaccine injection. It is increased by epinephrine injection. These reactions are in many cases the opposite of those produced in the skin.

Central nervous system.—In an excellent brief review of the cerebral circulation, Forbes emphasizes the general arterial pressure as the principal factor in determining the volume flow of blood through the brain. Dilatation of vessels, especially in response to carbon dioxide inhalation or to accumulation of metabolites in localized active areas, is the only functionally significant vasomotor reaction. The subject has also been reviewed by Malméjac.

The vasomotor responses of the cerebral vessels of cats and dogs to a number of drugs were found by Koopmans to be qualitatively similar to those exerted on blood vessels elsewhere in the body. Change in volume of the brain was used as the criterion of action. Metrazole, in doses adequate to produce convulsions, caused an active dilatation of pial arteries and veins unrelated to the convulsive seizures, according to Forbes & Nason.

According to Williams & Lennox, the cerebral blood flow in man, inferred from arteriovenous oxygen difference, is not abnormal in the presence of high intracranial pressure or arterial hypertension. Cerebral arteriosclerosis, in the absence of hypertension, may reduce the flow. A method for the recording of the blood flow in small cortical areas has been described by Jasper & Cipriani. This method has been applied by Jasper & Erickson to the study of the epileptiform discharge induced by electrical or chemical (metrazole or strychnine) stimulation of the cortex. The blood flow increased with simultaneous decrease in pH, both changes being attributed to the excessive neuronal activity.

The number of capillaries found to be open in various parts of the brain may depend upon the localization of action of the anesthetic employed. In animals under barbiturate anesthesia, Finley found greater vascularity in the supraoptic and paraventricular nuclei of the hypothalamus than in the cerebral cortex. Laidlaw & Kennard confirmed this observation, but found the converse to be true in ether anesthesia.

The caliber of the retinal blood vessels, photographed in dogs by Punttenney, decreases after mecholyl injection but usually increases after epinephrine. The literature on the reactions of retinal vessels is reviewed.

The claim that a pituitary-hypothalamic vascular connection

exists has been confirmed by Collin & Florentin. However, Brooks & Gersh have concluded that in the rabbit at least interruption of such connections plays no important part in the effects of section of the pituitary stalk, other sources of blood supply being adequate.

Skin.—A general review of the cutaneous circulation has been written by Malméjac. The methods of studying blood flow in the skin and the interpretation of the results have been reviewed by Burton (2) and by Ferris & Abramson (2). The latter emphasize the differentiation between arteriolar and venous reactions. Burton (3) has described a method for measuring peripheral blood flow. It depends upon changes in thermal conductance of the skin and is considered more reliable than methods dependent on skin temperature.

Selective vascular reactions occurring in different skin areas, the hands, feet, ears, nose, and forehead, have been studied by Hertzman & Dillon (1). Spontaneous waves of constriction or dilatation, differing in character and time relations, appear in these areas. Many stimuli, including auditory, psychic, and cold, cause vasoconstriction in the hands, feet, and nasal septum, but variable effects on the ear and head skin. Abramson & Ferris (1) found pinching, mental arithmetical calculation, and hyperventilation to cause the blood flow in the hands to decrease while that in the forearm increased or did not change. The skin of the hands differs from that of the forearm in having a greater number of capillaries and other vessels per unit area and in the presence of arteriovenous anastomoses.

The influence of smoking on the skin circulation has been studied by Abramson, Zazeela & Oppenheimer and by Franke & Hertzman. These workers agree that vasoconstriction and decreased flow result in the hands and feet; in other regions, such as the forearm or forehead, there is little if any change. The constriction is not entirely due to the reflex effect of deep inspiration, but may occur equally well in puffing on an unlighted cigarette. These results emphasize that the vascular responses of the skin of the hands are not typical of those of the skin of the body surface as a whole.

Reactive hyperemia in the hand, following a ten minute occlusion, was sufficient to repay only 10 to 20 per cent of the oxygen debt incurred, according to Abramson & Ferris (2); the maximal dilatation so produced is much less than that produced by the

local application of heat. In the forearm and leg, the reactive hyperemia fully compensated for the debt. Apparently the blood flow in these regions is closely related to the metabolic requirements of the tissue, while that in the hand depends primarily upon the needs for body heat dissipation.

Placenta.—Dawson & Robson have described a technique for perfusion of the fetal placental circulation through the umbilical artery. Contraction of the uterus momentarily decreases flow through the fetal placenta, but aids in the return of venous blood. Posterior pituitary extract or acetylcholine caused a sustained rise in fetal perfusion pressure, while epinephrine had no consistent effect.

PULMONARY CIRCULATION

An excellent review of the history and present status of knowledge of the pulmonary circulation has been written by Young; this subject has also been reviewed by Malméjac. Miyata has described pulmonary vessels, about 25μ in diameter, free from smooth muscle cells, lying between the arterioles and capillaries, and having elastic tissue continuous with that of the lung tissue. Di Natale has described a technique for microscopic study of the circulation in the frog's lung.

Hamilton (1) has discussed the dynamics of the pulmonary circuit. He believes that the blood vessels serve as a storage place for blood, being so distensible that their content can be several times normal without appreciable increase in pressure. This blood is available for mobilization in emergencies. He believes that there is no active physiological control of this function of the lungs. After a critical consideration of the experimental work on pulmonary vasomotor responses, nervous and chemical, he rejected all perfusion experiments on the basis of the pitifully small flow rates. He emphasizes that the only adequate criterion of an increase in pulmonary peripheral resistance is a greater gradient between pulmonary arterial and venous pressures. Since epinephrine either lessens this gradient or leaves it unchanged, while acetylcholine and amyl nitrite increase it slightly—these changes being opposite in direction to those to be expected from active vasomotor responses—Hamilton concludes that such vasomotor activity as there is in the pulmonary arterioles is without important function. Waele & Van de Velde (1), studying the influence of a number of procedures affecting the venous return to the right and left atria, found them

to produce relatively little effect on either pulmonary arterial or carotid pressure as long as the carotid sinus and depressor nerves were intact. They reached the general conclusion that the regulation of pressure in the lesser circulation occurred indirectly in the greater circulation. Steinitz & Friedberg, determining the pulmonary arterial pressure in trained unanesthetized dogs, found that pitressin caused a rise only when the left ventricle began to fail, epinephrine caused a rise, and acetylcholine had no effect. The authors believe that the arterial pressure changes result passively from difference in the outputs of the two ventricles.

In their study of the vasomotor responses of perfused lungs, Daly, Foggie & Hebb have taken advantage of the fact that the terminal branches of the bronchial arteries break up into capillaries which drain into the pulmonary veins to separate the actions of drugs on the pulmonary veins from those on the pulmonary arteries. Drugs introduced into the bronchial artery when the lungs are perfused in the normal direction reach only the pulmonary veins; when the perfusion is from the pulmonary veins into the pulmonary arteries, the action is confined to the latter. With this method, the authors have demonstrated that epinephrine constricts both arteries and veins.

The origin of the respiratory waves in the systemic arterial pressure has been studied by Trimby & Nicholson. Artificial reduction of the pressure on the external surface of the lungs, without alteration of that on other thoracic structures, increased the capacity of the vascular bed of the lungs and so reduced the filling of the left ventricle. This process is believed to be partly responsible for the inspiratory fall in arterial pressure normally seen, and is also believed to be important in delaying the rise in pressure resulting from increased filling of the right atrium during inspiration. Cahoon, Johnson & Michael have shown changes in the stroke volume of the ventricles consistent with the view of Trimby & Nicholson. The stroke volume and diastolic size of the ventricles decreased during inspiration. The right atrium increased while the left atrium decreased in size (as seen through chest wall windows). The blood content of the lungs also increased during inspiration.

CAPILLARIES

In a brief review of the histophysiology of the peripheral vascular beds, Knisely has discussed the relation of the three-dimen-

sional pattern of the capillaries in various organs to their function. Müller has published the second volume of his systematic treatise on the capillaries of the human skin in normal and pathological states. Clark & Clark have described the changes occurring during the regeneration of vessels studied microscopically in the living rabbit. Arteries, veins, and capillaries may change into each other by alteration of the number and arrangement of the extraendothelial cells, adventitial and muscular.

Methods for the measurement of capillary pressure have been studied by Eichna & Bordley. The direct micropipette method of Landis revealed the rise in capillary pressure produced by partial obstruction of the venous flow in the arm; the indirect pressure capsule method of Danzer & Hooker failed to detect it. The authors conclude that only the direct method gives credible results.

The flow of perfusion fluids through the capillaries of the frog's tongue or mesentery is much more nearly normal when the perfusion fluid contains particulate matter (India ink or red corpuscles) than when they are absent, according to an interesting report by Zweifach. Ringer's solution flows only through arterio-venous capillaries; the addition of particulate matter sets up swirls at the openings of the true capillaries, following which the axial current is deflected into these side vessels. The flow of fluid past the openings of capillaries may exert a suction on the capillaries; this may be a factor in controlling their diameter. The presence of particulate matter retards the development of edema in perfused tissues. Danielli has reported findings in agreement with those of Zweifach; the rate of development of edema in the perfused hind legs of frogs is retarded by the addition of serum, red cells, and platelets to a greater degree than can be accounted for by their colloidal osmotic pressure. The platelets are particularly effective, their action being largely mechanical, involving simple blockage of protein-permeable pores in the endothelial membrane. Achard has studied the applicability of Poiseuille's law to the flow of red corpuscle suspensions. He finds agreement when the pressure is not excessive; above a limit turbulence develops with slowing of the stream below the calculated value.

The permeability of capillaries in the tongue and mesentery of the frog has been studied by Chambers & Zweifach. They concluded that it depends largely on the intercellular cement which is spread over the luminal surface of the endothelial cells as well

as between them. It is continually being washed away by the blood and replaced by secretion by the endothelial cells. If the perfusion fluid is acid or lacks calcium, the cement softens and is removed more rapidly. These conditions, and also mechanical injury, favor extrusion of formed elements and outward diffusion of the fluid of the perfusate. The cement becomes sticky and red corpuscles adhere to the injured areas. Their role in plugging such leaks and so maintaining a normal permeability is emphasized by Zweifach. Rigdon has studied the time relations of the increased permeability of the capillaries of the rabbit's skin to trypan blue after xylol injury. Reactions of the capillaries in inflammation have been reviewed in the excellent monograph of Menkin (1).

Adrenal cortical hormone can prevent the increase in capillary permeability resulting from intracutaneous injection of leukotaxine or inflammatory exudate in the skin of the rabbit, according to Menkin (2). This finding supports the possibility previously suggested by others that the adrenal cortex may play a role in the regulation of capillary filtration.

SHOCK

The toxemic theory of shock, currently in disrepute, is once again revived by the results of Kendrick, Essex & Helmholtz. Blood from heart-lung-muscle preparations, the muscles of which had been traumatized, was injected into a recipient and an equal volume of the latter's blood transferred to the donor. After numerous such transfers, the arterial pressure of the recipient began to decrease and death occurred some hours later. If the muscles of the preparation were not traumatized, the recipient's pressure remained above the shock level and the animals survived. The results are considered suggestive, but not conclusive, evidence for the toxemic theory. Hyperthyroidism apparently predisposes to the development of traumatic shock, according to Schachter & Huntington. Davis has produced typical shock by subcutaneous injection of hypertonic sodium chloride solutions. Fluid is drawn osmotically to the injection site from the blood and interstitial spaces throughout the body. The blood volume reduction is adequate to produce shock.

Differences in the arterial pressure pulses in oligemic and neurogenic shock have been described by Hamilton (2). Due to the presence of vasoconstriction in the former type, waves reflected

from the arterioles are present in the pulse. These are absent in the latter type in which vasodilatation occurs.

Moon has emphasized the value of the measurement of hemoglobin concentration (by blood specific gravity, red count, hemoglobin, or hematocrit volume) in predicting the onset and estimating the severity of shock. In shock produced by a variety of means, hemoglobin concentration was invariably present, preceded the fall in arterial pressure, and was proportional to the apparent illness. Scudder has also urged its value.

CONCLUDING REMARKS

Certain general features of current advances in the study of the peripheral circulation may be indicated. While the most active single field is doubtless that of renal hypertension, there may be noted, among other topics, a growing interest in the participation of skeletal muscle tonus in vascular reflexes, in the possibility of physiological secretion of a pressor hormone by the posterior pituitary, and in the control of the blood flow in various regions of the skin. Particularly encouraging is the development of more precise methods for the study of the circulation in man and their application in the course of diagnostic and therapeutic procedures in the clinic. It is becoming clear that many accepted generalizations of circulatory physiology, developed largely by studies of the arterial pressure in anesthetized animals, must be reexamined in man with the aid of methods which take account of the complexity of vascular phenomena.

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ELECTRICAL ACTIVITY OF THE BRAIN

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Research on the electrical activity of the brain has made some progress since the review of Davis in the 1939 volume of this series (29) but it has received a very serious blow in the death of Joannes Gregorius Dusser de Barenne. The magnitude of this loss to neurophysiology has been dealt with elsewhere (53, 111) but since it is felt so keenly by those interested in the electrical activity of the brain it seems appropriate to pay tribute here to the outstanding work of one of the world's greatest investigators in this field.

Several reviews have appeared during the past two years, most of which have been primarily concerned with electroencephalography in man and its clinical applications (28, 30, 50, 69, 77, 78, 166). Especially significant are the comprehensive treatises of Bremer (17) and Bremer & Kleyntjens (20) and the review of Adrian in his Ferrier lecture before the Royal Society (2). The present brief review is intended to cover the period between September 1938 and September 1940 with particular reference to studies not included in the previous review of Davis.

EFFECTS OF LOCAL STIMULATION

Visual mechanisms.—The electrical response of the occipital cortex in the cat to visual stimulation was shown to be much more complicated without anaesthesia (in Bremer's "*l'encéphale isolé*" preparation) than has been previously described for animals under anaesthesia (22). A complex "on" effect was observed composed of a diphasic wave, the initial phase being positive, with amplitudes seven to eight times that of the background spontaneous activity. It was usually repeated four or five times then followed by a series of rapidly repeated waves at sixty to seventy per sec. The entire effect is of variable duration but may last for two to three seconds after the beginning of light stimulation.

Cessation of visual stimulation was marked by a cortical "off"

effect which was less constant, of simpler form, and appeared independent of the "on" mechanism as though arising from a different set of afferent neurons. Adaptation to light decreased the "on" effect and increased the "off" effect while adaptation to dark had the inverse result. Localization was restricted to the optic cortex (only so with bipolar leads) but appeared of identical form from both hemispheres with monocular illumination. Repeated stimulation (flicker) tended to reinforce the spontaneous rhythm of the cortex, as has been shown also in man (62, 63).

Careful correlative studies between detailed histological structure and localization, and pattern of cortical response to electrical stimulation of the optic nerve have been carried out on the anaesthetized rabbit (129). Responses were localized largely within the area striata of Rose except for some medial and anterior extension (128). Making certain assumptions, it was possible to attribute the initial positive wave to the termination of the afferent fibers of the optic radiation in the upper middle of the cortex while the second (negative) wave occupied the upper half of the cortex, suggesting impulse distribution via the plexiform layer. The third slow negative wave, identified with the alpha mechanism, was found in all layers, any deeper region being negative to the region above it. It was inferred that this final wave was due to impulses in the pyramidal cells of all layers whose axons leave the cortex by the basal fiber system.

Spontaneous alpha and beta waves were recorded from all depths of the optic cortex. It is pointed out that the "bewildering complexity" of histological structure presents as great a difficulty as the lack of detail in the "mass effect" obtained in cortical electrograms where units are buried in co-ordinate general activity. A very wide gap still exists between histological and physiological observations.

Dubner & Gerard (43) report spontaneous rhythms of different frequencies specific to the geniculate body as contrasted with cortical rhythms; the response of the geniculate to optic stimulation was also different in certain respects. Geniculate rhythms persisted with little alteration following extirpation of the occipital and parietal lobes, and following brain stem section caudal to the corpora quadrigemina. They were completely inhibited by light stimulation and depressed with nembutal. The geniculate "on" and "off" responses to light stimulation were described as diphasic,

consisting of an initial negative wave followed by a more prolonged positive wave which might be repeated rhythmically.

Continuing their illuminating analysis of the physiology of the optic system in the cat, Bishop & O'Leary (13) have traced the impulses from the optic nerve to lateral geniculate body and superior corpora quadrigemina. Four groups of fibers in the optic nerve were described, distinguished by four potential waves of different threshold and velocity of conduction. The most rapid wave was conducted to the geniculate body and cortex. The second, third, and fourth passed the geniculate body without synapse, the fourth, or slowest, conducting fiber giving a large response in the corpora quadrigemina.

After a single volley in the optic nerve, cortical activity showed a depression followed by facilitation having a phasic relation with the alpha rhythm. Response of the cortex followed repeated optic nerve stimulation up to one hundred per second. At first, amplitudes waxed and waned at the alpha-wave frequency in a manner similar to that in the observations previously reported by Talbot & Marshall (162). Finally, temporal dispersion of the alpha process with desynchronization of units occurred. This dispersion may explain continuous vision in the presence of a system which is undergoing rhythmic excitability changes at a low frequency (six per second).

The amplitude of geniculate response varied independently of that of the cortex; this fact, together with lack of synchronization in spontaneous rhythm, makes it improbable that the cortical alpha involves cortico-geniculo-cortical circuits. Geniculate facilitation seemed distinct from cortical facilitation.

Auditory mechanisms.—Large well-localized diphasic waves from the upper sylvian, the middle ectosylvian, and the posterior part of the anterior ectosylvian gyri in the unanaesthetized cat (using the method of "*l'encéphale isolé*") have been recorded in response to "click" stimuli (16, 18). There was close correspondence between the area giving such responses and that outlined from cytoarchitectonic studies. In all parts of this area, responses to pure tones of different frequencies were approximately equal; after fatigue with one tone, a response could be elicited with another.

Following the primary waves there was a more or less prolonged rhythmic after-discharge which radiated a considerable dis-

tance from the area of primary waves and was less constant. Strychnine increased extremely the primary wave either leaving the after-discharge unaffected or even suppressing it.

A still more generalized effect was observed in the desynchronization of spontaneous rhythms in widely separated cortical areas following a sound stimulus. Acceleration and increase in amplitude of spontaneous rhythms was also obtained under certain conditions. This type of generalized secondary response to auditory stimulation was previously reported in an unanaesthetized cat preparation without brain stem section in which the after-discharge was found to be a more localized response (142).

Potentials recorded from restricted areas of the medial geniculate body in the cat have been found to follow frequencies of sound stimulation up to about two thousand cycles (23). Maximum response for different frequencies was distributed in a fairly definite spacial pattern in the nucleus. It would appear from this that tones should be represented in spacial localization at the cortex, but experiments to date have failed to reveal any such cortical distribution.

Complex generalized electrical responses to auditory stimulation have been recorded in the electroencephalogram of human subjects asleep (33) and awake (32). A succession of rapid and slow waves ("K complex"), often rhythmic, was obtained from central and anterior head regions in response to various sounds and even to light and electric shock, administered when the subject was asleep. Latencies varied between 0.1 and 1.0 second. With the subject awake, a di- or triphasic wave ("on" and "off" effects) was obtained in response to "click" stimuli with a latency of about 30 to 40 msec. and appearing from the same general regions of the head as the "K complex" of sleep. These responses were not particularly prominent over temporal and occipital regions. They would appear "spontaneously" or by anticipation apparently dependent upon psychological factors similar to those affecting the alpha rhythm (7, 94, 173). They were in some respects quite independent of the alpha rhythm. This leaves uncertain the relationship between these evoked potentials and the alpha process, though they certainly represent a nonspecific generalized secondary response.

Vestibular mechanisms.—In confirmation of previous experiments with anaesthetized animals a cortical response to labyrinthine

thine stimulation has been obtained in the unanaesthetized cat (method of "*l'encéphale isolé*") (57). As for auditory stimulation, a primary local cortical and a secondary generalized response was obtained. The primary response was confined to the suprasylvian gyrus. Impulses were followed from the lateral lemniscus to the posterior corpus quadrigeminum and the medial geniculate body to the cortex. The secondary response was obtained from all available cortical areas and consisted of an increase in frequency and amplitude and regularization of spontaneous activity. It was considered due to a general awakening of the animal or increase in the "*tonus corticale*."

Vestibular stimulation in man (by irrigation and rotation) was found to result in a suppressing of the alpha rhythm with rapid waves (twenty to forty per second) followed by slow base-line swings appearing several minutes after stimulation (109).

Tactual mechanisms.—Local cortical potentials in response to tactual stimulation of cat's paws have revealed a double representation with two areas for the hind foot two centimeters apart. The second representation was found behind the head area in the ectosylvian gyrus. It was not present in the dog (1).

Peripheral nerve stimulation.—Analysis of cortical response to sciatic nerve stimulation in the cat under deep barbiturate anaesthesia (51) has revealed a distinct localized primary response apparently coincident with the arrival of a volley of afferent impulses at the cerebrum (with latency of 10 to 12 msec.). A more generalized slower secondary response (with latency of 40 to 80 msec.) followed a single primary response but was extinguished with repeated stimuli at rates greater than three per second. The secondary response would not appear shortly after a spontaneous wave. It appeared with about the same latency and duration in both hemispheres and in regions remote from the sensory area where the primary response was strictly localized. The secondary response was thought to be elaborated in the thalamus before reaching the cortex and to involve the same mechanism as that causing the spontaneous alpha rhythm in the unanaesthetized animal.

The immediate response of the sensorimotor cortex to electrical stimulation of the isolated saphenous nerve (under ether or pentobarbital anaesthesia) was a series of three waves which, by their threshold and conduction velocity were identified with the alpha,

delta, and C fibers of the cat's saphenous nerve (70). Cyclic facilitation developed at the frequencies of the spontaneous alpha rhythm.

Stimulation of the cerebral cortex.—In the anaesthetized animal, local electrical stimulation of one hemisphere resulted in a local potential wave from the homologous area of the contralateral cortex, which was abolished by section of the corpus callosum (25, 26). This contralateral response was diphasic with an initial surface-positive wave appearing in 15 msec. followed in 75 msec. by a surface-negative wave. Picrotoxin increased the negative wave with little effect on the positive wave, while pentobarbital abolished the negative wave, leaving the positive one unaffected.

Studies with microelectrodes at different levels in the cortex suggested that the surface-positive wave was due to impulses in ascending callosal fibers, while the negative component seemed related to internuncials. Contralateral waves could be evoked from all areas except the operculum of the occipital lobe. They were associated with facilitation, inhibition, or no change in local excitability (25). In unanaesthetized or lightly anaesthetized animals, local after-discharge followed electrical stimulation in homolateral and local contralateral motor areas (123, 124). It was altered in form but not abolished by section of the corpus callosum (49).

Local surface-negative waves appeared at the site of stimulation with short weak stimuli, but became surface-positive and spread with a surface velocity of 5 to 50 cm. per sec. when deeper layers were involved with longer and stronger stimuli (3). The spread was diminished with anaesthesia. After-discharges especially showed a wide spread with light anaesthesia.

Dusser de Barenne & McCulloch have given an excellent summary and theoretical treatment of their extensive fundamental studies on the electrical properties of the cerebral cortex in relation to electrical excitability and local pH (46), which cannot be given the space it deserves in this review. In brief, factors for facilitation considered were (a) hyperactivity, as shown by increased electrical activity in after-discharge following electrical stimulation resulting in a central (reverberating circuit) summation, (b) negative direct current voltage drift associated with decrease in threshold of neurons involved, and (c) probably an increase in pH (alkaline shift) which serves to decrease the threshold of all neural structures in the region involved. Extinction, following the period of

facilitation, was associated with reverse changes in all three variables.

Some complication in this scheme is introduced by the possibility of direct current artifacts in the pH record and by the observation that local cerebral blood flow is also increased with facilitation and that this causes an alkaline shift in pH (85, 86). Also, Adrian & Moruzzi (4) consider the period of depressed activity following facilitation to be a "fatigue" process due to excessive neuronal discharge, probably not physiological, since it did not occur with stimuli, subliminal for motor response, but which produced minimal facilitation as judged by changes in the cortical electrogram.

Moruzzi (123) found that weak cortical stimuli in the unanesthetized animal produced a decrease in amplitude with a marked increase in frequency in both homolateral and contralateral cortical areas and that this decrease in amplitude was associated with facilitation as judged by lowered threshold to electrical stimulation for motor response, and was not followed by "extinction." Increased intensity of stimulation caused large epileptiform waves, an excessive synchronized discharge which was followed by a depression of electrical activity and of cortical excitability ("extinction").

It seems clear that the long after-discharges following strong electrical stimulation to the cortex are more closely related to epilepsy than to normal cortical function. However, the clarity of pathological conditions often teaches much regarding physiological processes.

Cerebellar stimulation.—Weak faradic stimulation applied to the cerebellar cortex was followed by a period of diminished electrical activity at the site of stimulation, but with longer and stronger stimuli there was marked augmentation of activity with increased amplitude and better synchronization. Frequencies increased to 300 per sec. (36, 37). This in turn was followed by a much longer period of diminished activity. These local changes were not observed 4 mm. from the site of derivation which is in keeping with the absence of long association tracts.

Stimulation of the various afferent connections to the cerebellum produced local response only in the neurons of the cerebellar cortex with which the respective afferent fiber systems are directly connected. Little effect was observed upon the background spontaneous activity except that occasionally it was initiated or in-

creased, but never decreased. Strychnine applied locally to the cerebellar cortex produced no spikes and generally little effect except a depression of activity.

Diencephalic stimulation.—Stimulation and recording of electrical activity from cerebral structures in the vicinity of the body of the sphenoid bone, presumably hypothalamic, by an electrode placed in contact with this bone in the posterior nasopharynx, has been carried out in man and animals (65). "Hypothalamic" electrograms were found to differ in predominant frequency from those of the cerebral cortex in regard to spontaneous activity. They also showed differential reactions to electrical and "emotional" stimulation and to various drugs. "Hypothalamic" stimulation was found to alter cortical rhythms, this fact suggesting a "driving" of the cortex by the hypothalamus.

From studies employing direct monopolar recording from different areas of the hypothalamus in dogs, it was concluded that in the indirect method potentials are led mainly from the anterior hypothalamus, since anterior and posterior regions showed different reactions to hypoglycemia and to various drugs (75).

Strychnine spikes.—Strychnine spikes are included here because of their continued value, especially in the hands of Dusser de Barenne & McCulloch (44, 45, 47) and others (15, 90), in the delimitation of tracts and functionally related cortical areas. Local application of minimal doses apparently "fires" only those cells with which it comes into immediate contact, the resulting excitation not crossing synapse, so that the electrical tracing of the impulse in this fiber provides rapid "anatomical" localization. Examples of its use are the discovery of a direct fiber tract from the nuclei cuneatus and gracilis to the sensory cortex and another ending in the globus pallidus. Its further value in cortical localization is illustrated by the mapping of the sensory cortex in the chimpanzee brain (6). Such localization studies must be interpreted in the light of the method employed.

The fact that, in addition to "firing," suppression of activity in one area may result from strychninization of another even when the strychninized area lies outside the sensory cortex proper (54) raises some question as to the limitation of the action of strychnine to only those neurons directly affected. Sufficient local strychninization will certainly set up impulses which cross synapses, though well-restricted local effects are the first to appear (4, 125).

Local spikes with properties similar to those of the strychnine spikes have been found characteristic of local cortical areas in man giving rise to focal epilepsy (83, 89, 135).

Analysis of the action of strychnine on the nervous system has led to the conclusion that effects are produced principally by alterations in excitability and accommodation of cells, especially those of the central nervous system, and that this drug has only a minimal questionable effect in lowering synaptic thresholds (71). A marked decrease in accommodation, counteracted by the reverse action of calcium, was considered the principal action.

Caffeine waves.—Waves of large amplitude (0.5 to 1.0 mv.), of 0.1 to 0.2 second duration, have been recorded from the isolated cortex of the frog following the local application of caffeine and have been shown to have some remarkable characteristics which (when further verified) may be of fundamental importance (55, 56). These waves were apparently conducted across the cortex with surface velocities of 4 to 22 cm. per sec., the rate being dependent upon temperature. The remarkable observation is that they seem to pass from a focus to an adjacent area when synapses had been blocked with nicotine and even when the two areas were completely separated by clean section. If confirmed this observation indicates that, for large amplitude discharges at least, activity may spread asynchronously from one cortical area to another by successive electrical stimulation by action potentials from adjacent neurons.

GENERAL FACTORS AFFECTING SPONTANEOUS CORTICAL RHYTHMS

Interrelationship between various factors controlling the spontaneous activity of the cortex has been presented by Gerard and associates (55, 56, 102) and Bremer and associates (17). Only a brief account of a few of these factors can be given here. In general, increased frequency accompanies excitatory agents and decreased frequency depressing agents (151), but this scheme is complicated by changes in synchronization ("trip mechanism").

Anaesthesia.—Studies of the electrical activity of various brain regions promises to yield much of value regarding the nature of anaesthetic action. All anaesthetics in adequate doses seem to have some effect on all parts of the brain, there being none found which affected only higher as opposed to lower levels (38, 41, 42). Never-

theless, different degrees of action in different areas or levels are shown by differential effects of small or large doses, and by extirpation studies (70, 152).

Careful, comprehensive, and detailed analysis of the cortical electrograms of cats subjected to seventeen different kinds of anaesthesia induced by agents varying from the most volatile (nitrous oxide, cyclopropane, etc.) to nonvolatile anaesthetics (pentobarbital, chloralose, etc.) have been made at two specific levels of anaesthesia as tested by response to sciatic nerve stimulation (12). In general, the frequency of cortical activity was constant for a given anaesthetic through a wide range of depths of anaesthesia but the amplitude varied widely both with kind and depth of anaesthesia. Higher frequencies characterized anaesthesia with the volatile agents, while lower frequencies were obtained with nonvolatile agents. [See also Drohocki & Drohocka (40).] These two groups were separated consistently on the basis of several criteria: molecular size, volatility, frequency per second of cortical waves, voltage, pattern, presence or absence of secondary discharge following sciatic stimulation, type of flexor response to sciatic stimulation, and ability of sciatic stimulation to alter the voltage of cortical waves under light anaesthesia. A much more complicated effect with slow frequencies under a highly volatile anaesthetic has been found in man (147).

The increase in frequency with ether or urethane as compared with the slowing with pentobarbital has been corroborated once more (4, 40) in addition to a demonstration of differential effects upon the cortical response to alpha, delta, and C fibers of the saphenous nerve, these waves disappearing in the reverse order with increasing depth of anaesthesia (70). This is in keeping with the common observation that pain sensibility is lost before touch. The primary action of ether was found to be a blocking of conduction at the synapse before responsiveness of cells beyond it were greatly affected. This explains the decrease in general facilitation and spread.

With sufficient ether to cause a drop in amplitude of cortical activity, pH studies have shown local cortical acidity which returns to normal with the return of electrical activity (126).

In a study of the effect of eleven different alcohols, cortical potential frequency was found to become progressively slower with increased molecular weight. The relationship was shown to be de-

pendent upon anaesthetic potency rather than on molecular weight as such (11).

Electrical factors.—The importance of purely electrical factors in the control of cortical activity is brought out most clearly in the work of Gerard & Libet (55). These factors may operate by the setting up of direct current voltage gradients (polarization) across large cell masses. Such gradients do occur "spontaneously" in relation to cortical activity (46). They may also operate by the effect of local action potentials themselves upon adjacent neurons. With regard to polarization, it is important that the cathodal region is depressed during current flow, this being the opposite of effects on nerve. The precise mechanism involved is in need of further clarification.

Metabolic factors.—There is apparently a marked parallelism in the effects of hypoxia and hypoglycemia upon the spontaneous activity of the cortex. In lightly anaesthetized or unanaesthetized animals (43, 123, 150) and even in isolated crayfish ganglia (139), the first effects are shown by marked increase in electrical activity (facilitation) although only a depression of activity was observed by some investigators (64). This excitation phase may be followed by large slow waves before complete depression of all activity occurs. The depressed phase may be interrupted at intervals by large amplitude epileptiform waves before complete disappearance of all electrical activity (123). This depression of activity does not appear to be due to interruption of synapses, for synaptic conduction is retained or even enhanced when the electrical activity has completely disappeared from the cortex.

Studies with the human electroencephalogram have not revealed the phase of increased activity but they have shown the presence of slow waves (24), either appearing rather abruptly at critical levels (61) or with gradually decreasing frequency which shows a direct relationship to cerebral oxygen consumption (72, 73, 74).

Increased metabolism, such as is produced by large injections of methylene blue (123), by sodium cyanide (148), or dinitrophenol (76), have a facilitating effect on amplitude and frequency of cortical activity (with certain interesting exceptions on sodium cyanide injection into patients with pathological conditions of the brain). Vitamin-B deficiency caused slow waves to appear in the pigeon's cortical electrogram, these waves promptly disappearing with

thiamin injection (165). The relationship with oxidative enzyme systems is given an interesting theoretical treatment by Hoagland *et al.* (67, 68).

A marked discrepancy has appeared between those carbohydrates capable of maintaining normal electrical activity and those metabolized by isolated brain tissue (115).

Ion concentration.—The spontaneous activity of the crayfish ganglion showed little change with hydrogen-ion concentration over a moderate range, but with extremely low concentrations (pH 8) the activity was decreased, while with high concentrations the activity was increased (138). This is the reverse of the relationships reported for the mammalian cortex (48) over a small pH range but more comparable to certain effects caused by more extreme changes (43). The human electroencephalogram shows a marked sensitivity to pH especially in patients with epilepsy (60, 61, 127).

Potassium is of particular significance in controlling the spontaneous discharge of crayfish ganglion cells (137, 138), the magnitude of spontaneous discharge being in inverse proportion to the extracellular potassium concentration. Similar results with potassium are reported for the mammalian cortex (43, 102); important effects of sodium, calcium, etc., are also described.

Neural factors.—The dependence of normal cortical electrograms upon intact fiber connections with certain subcortical projection nuclei has received further demonstration by experimental tract section (13, 17, 46, 113) and by pathological lesions involving the optic radiations in man (9, 21). Section of commissural tracts as well may cause marked alterations in the character of cortical rhythms in the two hemispheres (22).

Acetylcholine and metrazole.—The facilitating effect of small doses of acetylcholine upon the electrical activity of the cerebral cortex appears similar to that caused directly by electrical stimulation, and indirectly by nerve impulses (20, 122, 124, 125) or by metrazol (64). A depression of activity may occur with large doses.

The effect of metrazole on the human electroencephalogram has been extensively studied through the opportunities afforded by its use in certain forms of psychiatric treatment (34, 149, 159).

Cerebral circulation, edema and intracranial pressure.—Cerebral anaemia produces changes in the electrical activity of the cortex

similar to those described for hypoxia and hypoglycemia, complicated by effects of hypercapnia and increased extracellular potassium (150). Complete arterial occlusion in the anaesthetized cat caused a disappearance of fast waves with appearance of slow waves in ten to twelve seconds all activity having disappeared in about twenty seconds, the higher neural levels dropping out first. An initial period of facilitation of one to two seconds after circulatory arrest is also occasionally observed. The cerebellum appears particularly sensitive to anaemia (36). Unilateral carotid ligation in man may cause slow waves to appear in the electroencephalogram of the affected side and these changes appeared to be completely reversible (176).

Increased intracranial pressure as such appears to have little effect on cortical electrograms but generalized delta waves with absence of normal rhythm appear with cerebral edema (75, 174).

Relation to cytoarchitectural structure.—The original findings of Kornmüller (96) which suggested a definite sharply localized relationship between the pattern of bioelectric activity and cortical areas mapped out according to cytoarchitectonic structure (Vogt) have not received detailed confirmation (5, 10, 39, 108, 142) although some distinguishing features of the complex patterns obtained seem related to functionally homogeneous areas. Working with unanaesthetized animals, some investigators have found that fundamental spontaneous rhythms from the entire cortex are more dependent upon general metabolic changes than upon cytoarchitectonic structure but that certain functional changes in a given system (such as the acoustic or optic) may cause local differences to appear (119).

Cerebral structures as different as those in the pigeon and rabbit may produce similar electrograms (19).

Microelectrode studies have shown that certain elements of cortical activity may be related to specific cell layers (25, 26, 129, 141).

Further evidence has confirmed the finding that the alpha rhythm is not confined to the occipital lobes (104, 166, 172). The beta rhythm seems more prominent from precentral regions (121) where it has been attributed to layers 3 and 5, since it disappears in patients with amyotrophic lateral sclerosis. Homologous areas of the two hemispheres in man have been shown to have very similar

electrograms while significant differences exist between widely separated areas (163). Interrelationships exist, however, between all areas.

A certain dependence upon structure (or possibly function) is shown in the changes with age (103, 106) and when grossly different structures are compared such as the cerebellar and cerebral cortices (36). The principal difficulty seems to be in differentiating between functional states, to which the electrogram is most sensitive, and cytoarchitectonic structure as such.

Heredity and lateral dominance.—Confirmation of the similarity of electroencephalograms in identical twins has appeared (140), together with a demonstration of interesting asymmetries between the two hemispheres related to lateral dominance (handedness).

RELATIONS WITH THE EFFERENT SYSTEMS

A most fundamental contribution to our knowledge of efferent relations with cortical waves has been made by Adrian & Moruzzi (4). Bursts of rapid axon spikes appeared in the pyramidal tracts at the level of the decussation at times corresponding to the slow waves from the motor cortex. The spontaneous cortical rhythm under dial at a rate of seven to ten per second was associated with synchronized bursts of axon spikes. The cortical waves occurred without tract spikes in anaemia. Electrical stimulation and convulsant drugs produced a marked increase in the frequency of spikes in each burst, up to over one thousand per second, related to increased amplitude of cortical waves. Motor response appeared only with the more rapid repeated discharge in pyramidal fibers associated with larger cortical waves [See also Moruzzi (125)].

Other investigators have observed definite relationships between potential waves in the cord and cortex (15), in the basal ganglia and motor cortex (90), between the electrical activity of the cerebellum and muscle tone (36), that of the cortex and the knee jerk (112), and between the electroencephalogram and muscle tone in man (14).

CLINICAL STUDIES

Localization of cerebral lesions.—The electroencephalogram has become of importance comparable to the pneumoencephalogram as an aid in the diagnosis and localization of cerebral lesions, as has been shown by numerous investigators (8, 81, 82, 83, 87, 96, 97, 99,

116, 118, 120, 143, 155, 156, 168, 169, 170, 174, 175). This method depends chiefly upon the presence of "delta foci," slow waves with a rate of between less than one and six per second, which arise from cerebral tissue, usually adjacent to the lesion proper, which is in an active pathological state probably related to local edema, impaired circulation, or active degenerative processes. Experimental studies have shown delta waves, in addition to more complex discharges, following lead intoxication (157) or x-ray irradiation of the brain (35).

Stationary or very benign lesions do not give rise to delta waves but may sometimes be localized by the absence of electrical activity immediately over them ("quiet regions"). Local lesions giving rise to epileptogenic discharge have specific forms (spikes and sharp waves) in addition to the delta waves. It is hoped that when another such review is written it will be possible to make some definite statements regarding the neurophysiological mechanism underlying these clinically useful electrical waves.

Epilepsy.—The clear, often dramatic, changes in the electrical activity of the brain in epileptic disorders makes this a most fruitful field for the application of electroencephalography. Numerous clinical studies of epilepsy and allied disorders have appeared during the past two years which cannot be adequately treated here but which merit careful study because of the interesting physiological problems presented and the occasional ray of light shed upon normal cerebral function (59, 60, 61, 66, 89, 91, 92, 93, 127, 130, 131, 132, 153, 158, 167).

The discovery of certain forms of abnormality in the electroencephalograms of children with severe ("epileptoid") behaviour problems (88) has received confirmation (27, 58, 107, 161), so illustrating the tendency for broadening of the fringe of epilepsy to invade a variety of clinical categories. Electrographic abnormalities of the same form (but less severe) as those observed in "essential" or "idiopathic" epileptics have been obtained in a relatively high percentage of their clinically normal relatives (101, 110, 160). This raises important problems regarding the heredity of epilepsy as well as the significance of such electrographic disorders if accompanied by no obvious clinical abnormality.

Electroencephalograms in epilepsy have received different, though not wholly opposed, theoretical interpretations. Gibbs & Lennox (58, 59, 60) relate epileptic discharge to generalized meta-

bolic disorders (involving oxygen, carbon dioxide, sugar, etc.) affecting the rate regulators of neuronal discharge, a "dysrhythmia." This view has the support of such physiological studies as those of Hoagland (73, 74) on "chemical pacemakers." Jasper maintains (83, 89) that the rate of neuronal discharge is of relatively minor importance (since the onset of seizures may occur without the appearance of abnormal rhythms) but that the fundamental disorder is related to factors producing excessive facilitation with synchronous discharge of large masses of neurons or "hypersynchrony." This point of view has the support of such physiological studies as those of Adrian (4), Bremer (17), and Moruzzi (125). According to the former view, different clinical forms of seizure are due mainly to different frequencies of discharge in the brain as a whole. The latter view would explain different forms of clinical seizure upon the basis of the physiological function of the local brain area primarily involved and its functional relationships.

Relations with states of consciousness.—Further studies of the human electroencephalogram during normal sleep have confirmed and clarified in greater detail the close relationship with depth of sleep described qualitatively by previous investigators (14, 79, 95, 117). Delta waves appeared at rather higher levels of apparent consciousness in narcolepsy and in hypersomnia (due to prolonged insomnia). These waves were decreased by amphetamine and increased by alcohol.

The electroencephalogram during narcoleptic sleep was found similar to that of normal sleep but distinct in some respects from that obtained in other forms of unconsciousness such as coma or epileptic states (80, 117, 154). The clinical syndrome of consciousness may be therefore related to quite different conditions of cerebral physiology. Pathological sleep due to hypothalamic lesions, however, may reproduce quite faithfully the conditions of normal sleep as judged by similarities in the electroencephalogram (171).

Psychiatric disorders.—Electroencephalograms from patients with mental disease show a wide variety of abnormalities as well as many within normal limits (31, 52, 67, 84, 100, 105, 114, 133, 136, 143, 144, 146). Except for the discovery of unsuspected localized lesions results are, for the most part, too complicated for present interpretation and show little if any relationship to current diagnostic categories. Equally complicated are relations to mental

deficiency (98). It is of practical importance that the purely psychological disorders (psychoneuroses, hysterias, etc.) generally have electroencephalograms within normal functional limits (134), although hysterical hemianaesthesia may block the normal electrical response of the cortex to strong stimulation of the affected side (164).

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THE AUTONOMIC NERVOUS SYSTEM

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Since Langley's time it has become customary to restrict the term autonomic nervous system to efferent neurons only. This leaves the afferent fibers found in autonomic nerves in somewhat an ambiguous position from the point of view of classification. Anatomically they are generally believed to be identical with somatic afferent neurons, except that they arise from nerve endings within visceral structures (i.e., smooth muscle and glands) and run for part of their course with the autonomic efferents; physiologically, a distinction between visceral and somatic afferents is still disputed. There is therefore much to be said in favor of Livingston's classification (210) of a visceral nervous system comprising afferent and efferent neurons, the latter (under the term "autonomic") being divisible, as Langley first suggested, into a sympathetic and a parasympathetic division. These terms, sympathetic and parasympathetic, have through usage come to be regarded as synonymous with the thoracolumbar and craniosacral outflows respectively, and although when used in a physiological sense they have general meaning, as Cannon showed in his studies of emotional behavior, yet confusion in thought has occurred, for fibers producing both augmentory and inhibitory effects in a single organ run in each outflow. Dale's suggestion that individual neurons, whether in the thoracolumbar or craniosacral outflows, be referred to as adrenergic or cholinergic, is based on the distinction between chemical substances produced at the termination of the axons. As applied to postganglionic neurons such terms have additional physiological meaning in that the types of response in the effector can often be predicted. Despite custom, which has been followed in this review, it is not strictly correct to speak of augmentory and inhibitory "neurons." The neuron is a conductor of an impulse, the response to which depends on many factors at the synapse or at the neuromuscular junction.

Sympathetic and parasympathetic have too long been used to mean bundles of nerve fibers or groups of ganglion cells, and it is in this anatomical sense that these terms will be used in this review.

We are accustomed to speak of sympathetic and parasympathetic "discharge" in response to experimental excitation within the central nervous system. It might be better here to use the common term autonomic or to refer specifically to the type of response elicited. In the matter of terminology in the field of the visceral nervous system too many names have been adopted and too few definitions followed.

VISCERAL AFFERENTS

In the past year little new has been added to our general concept of the anatomy of the visceral afferent neurons. The belief persists that most, if not all, have their cell stations in dorsal spinal root ganglia or in the corresponding sensory ganglia of the cranial nerves. Palumbi (250) found in sympathetic ganglia no cells of the type found in spinal ganglia. Nevertheless, certain authors, Leriche, Danielopolu, and Kuntz, still believe in the existence of some visceral afferent cells lying within autonomic ganglia, and in the possibility of local reflexes through such peripherally placed synaptic connections. Kuntz (184, 185) has added new evidence in support of this view. Gastrointestinal reflexes have been demonstrated following the complete functional separation of the celiac and of the inferior mesenteric ganglia from the central nervous system, and elimination of the enteric plexus as a factor. It would be more convincing if removal of the ganglia is found to abolish the particular reflexes in question. Histological degeneration studies supported Kuntz's assumption that axons of enteric origin affect synaptic connections with cells in the celiac and inferior mesenteric ganglia, although it is very difficult to be certain that all preganglionic fibers to these ganglia had been divided in the experiments. An open mind must be kept on this problem until further facts be forthcoming.

The exact pathways and points of entry into the cerebrospinal axis of visceral afferents from particular organs are still too vaguely understood. Clinically, from the standpoint of interrupting pain pathways, a detailed anatomy of the visceral afferents is becoming increasingly important. It cannot be assumed that they always enter the cerebrospinal axis at the same levels from which the corresponding efferents emerge. Fay (85), for example, believes that pathways conveying vascular pain from the inferior extremity enter the spinal cord in the dorsal roots of T2 to T5 (inclu-

sive), well above the outflow of sympathetic fibers to the leg. He bases this view on the observation that, after complete spinal anesthesia up to the level of T6, pain in an amputation stump of the lower limb can persist, but that when the spinal anesthesia is carried up to the level of T2 the pain may be completely abolished. Chordotomy at this high level in certain cases was followed by a permanent relief of pain.

More light has been thrown on the question of physiological differences between visceral and somatic afferents. Leriche (197) and Morley (242) both distinguished clearly between "visceral pain" and "pain of cerebro-spinal type." The accurately localized, sharp pain of the skin in response to pricking or cutting was contrasted with the diffuse, vaguely localized pain of visceral origin, with its tendency to be referred to distant areas of the body surface and its peculiarly unbearable quality in its severest form. Any such fundamental difference between the two systems of afferents is questioned however by Lewis & Kellgren (200) from studies of the pain response of deeply placed somatic structures, such as deep fascia, muscles, and ligaments. Pain derived from deep fascia by the injection of hypertonic saline into its layers is localized with fair accuracy but not as sharply as that evoked from the skin (176). Pain from muscle, whether produced by working the muscle under ischemic conditions or by the injection of small quantities of hypertonic saline,¹ is invariably of an aching and disagreeable quality. It is diffuse, difficult to localize, and often referred to a distance (198). The pain from muscle does not appear to be referred to areas of skin, but rather to deep structures, and is associated with deep tenderness. The pattern of reference corresponds not so much with the sensory segmental zones of the skin, as worked out by Head and Foerster, as with the spinal segments from which the motor innervation of the muscle is derived. It may be argued that such radiation of pain cannot be compared with reference from one type of tissue to another, but Lewis & Kellgren believe there exists a gradual transition from pain which is confined to the structure stimulated to diffuse pain of a segmental distribution, finally referred by other segments to a distance. The degree

¹ The reason why hypertonic saline injected into a muscle belly produces a pain identical in quality with that caused by ischemic work has been studied by Maison (222), in an attempt to find the muscle catabolite which causes the pain, as yet, however, the studies are inconclusive.

of localization in their opinion depends on the depth at which the structure lies rather than on its nature. Injections of hypertonic saline into the interspinous ligaments in man for example gave rise to pain, tenderness, and tonic contraction of the muscles of the trunk, having appropriate segmental distributions, phenomena identical with those associated with visceral disease in man. In the decapitated cat the reflex muscular contractions of the abdominal wall, provoked when the spinal muscles or the pancreas were stimulated appropriately, were indistinguishable (200). In this Lewis & Kellgren are in part agreement with Morley (242), who showed that in abdominal disease pain referred to distant points is derived not from visceral but from somatic structures, but they depart from Morley's theory in that they do not accept visceral pain as in any way specific.

Whether there is any fundamental distinction between somatic and visceral afferent neurons is uncertain, but the tendency seems to be away from such a belief. There is clinical evidence that the viscera may be directly sensitive to pressure if it is applied adequately, and particularly under certain pathological conditions (178, 197). Morley's theory that impulses derived from abdominal viscera do not give rise to referred pain and tenderness, nor to reflex muscular rigidity, unless the parietal peritoneum is involved, does not appear to hold when applied to other visceral structures such as the heart. Even in the abdomen Kinsella (178) has made clinical observations which cast some doubt on its universal application. Nevertheless the importance for diagnosis of the pain reference and muscular reflexes from parietal peritoneum cannot be gainsaid, and much of the evidence supposedly in disagreement with this view of Morley is open to other interpretations. Vasoconstrictor reflexes in the toes and fingers, for example, followed sudden dilatation of the duodenum experimentally (45), and Sturup's (299) and Jones' (168) investigations of the sensations produced in normal individuals when the esophagus was distended by a balloon revealed reference of pain, hyperalgesic areas, and Wernoe's zones of vasoconstriction. It must be pointed out, however, that in such experiments on extraperitoneal viscera, such as the esophagus, and on retroperitoneal parts of the gut, such as the duodenum, sudden distension must inevitably cause tension on the loose areolar tissue surrounding the viscus, and the results cannot therefore be interpreted with certainty as being due to visceral stimulation

only. Similarly, studies of Layne & Bergh (194) on distension of the common bile duct in unanesthetized human subjects demonstrated in some patients reference of pain to the right subscapular area, which they interpreted as evidence of a viscerosensory radiation, but one must not forget that the biliary tract receives somatic afferent fibers via the phrenic nerve, a fact recently confirmed by Alexander (2). That referred pain produced by distension of a balloon in the upper part of the small intestine can no longer be felt after section of the splanchnic nerves (19a, 318) is significant for it does indicate a mechanism of reference via visceral afferent fibers.

Hinsey, Hare & Phillips (153) have added some interesting observations upon diaphragmatic sensibility and upon the afferent components in the phrenic nerve. In opposition to the concept of Pollack & Davis of viscerocutaneous reflexes in explanation of the shoulder-tip pain from subdiaphragmatic stimulation, Hinsey & Phillips (154) have found that nociceptive sensibility produced by stimulation of the central portion of the diaphragmatic peritoneum in the cat and dog depends only upon afferent conduction in the phrenic nerve and is present after bilateral section of the vagi, bilateral excision of the sympathetic chains from above the superior cervical ganglion through T7, and after transection of the spinal cord at T3 or T4.

The concept of an "irritable focus" in the cord, so difficult to grasp on account of its essential vagueness, and the mechanism of visceromotor and somatomotor reflexes become clear "if we assume that both somatic and visceral afferent fibres carry impulses which affect a common pool of secondary neurones, and that the principles of summation and inhibition are applicable" (154). Such an interpretation of reference of pain utilizes physiological terms with which we are familiar in our understanding of the synapse. Facilitation through education may decide the site of localization. Thus children do not localize pain as accurately as do adults, and the blind acquire a power of localization far beyond that of the average man. Likewise the patient visited repeatedly by similar attacks of abdominal disease will in time come to localize the pain remarkably accurately (Leriche). The reference of pain to the distribution of only one nerve of the same spinal segment, or of one branch of a nerve rather than another (such as the anterior rather than the posterior division) may be merely an anatomical question of the

central connections of the various afferent neurons (19a). Thus the sensory fibers from the dome of the diaphragm may terminate around cells in the dorsal column of grey matter around which only fibers from the shoulder tip have endings. On this basis alterations of the site of stimulation on the lower surface of the diaphragm should change the area of referred pain, a fact which has recently been demonstrated by Livingston [see Hinsey & Phillips (154)]. The importance of inhibitory and excitatory impulses from higher centers on such an "irritable focus" in the cord, has recently been emphasized by Kinsella (179).

In referring to the afferent pathways for pain conduction from muscles and fascia as somatic, Lewis & Kellgren (200) omit any special consideration of the afferents accompanying the blood vessels, which are visceral in that they arise from visceral structures (smooth muscle) and accompany autonomic fibers on their way to the cerebrospinal axis. Leriche (197) has repeatedly emphasized the diffuse localization and unbearable character of vascular pain "of sympathetic origin." It cannot be due entirely to local vascular changes altering the threshold of somatic afferent nerve endings, though this undoubtedly plays an important role. One recalls Fay's observation (85), referred to above, of pain from an amputation stump of the leg persisting after complete spinal anesthesia up to the level of T6 and its disappearance when the anesthetic was carried up to the level of T2; one also recalls a case reported by Slaughtner (288) in which a complete transection of the spinal cord at the level of the first lumbar segment (confirmed by operation) was followed by pain in both lower extremities, which was relieved only by bilateral lumbar sympathectomy. Electrical stimulation of the median nerve in the dog still caused reflex hypertension when the brachial plexus was sectioned, but not if the subclavian artery was denervated and the stellate ganglion removed (307). Leriche has long held to the view that, in vascular occlusion, the irritative process initiates reflexes via afferent neurons from the vessel wall, which give rise to peripheral vasospasm, edema, and even to muscular spasm in the affected extremity. The value of excision of the occluded vessel in such conditions, which Leriche strongly advocates, has now been confirmed by Homans (160) and by Ochsner & DeBailey (247).

Over a period of years now Livingston (211, 212, 213) has made a special study of so-called "intractable" pain. Post-traumatic

pain syndromes, back injuries, true causalgias, and phantom limb cases have come under close observation. The clinical picture in most of these conditions is characterized by unbearable pain, often referred, and associated frequently with acute hyperesthesia, edema (159), and trophic changes. Many show spasm of the voluntary muscles. The similarity to pain from the abdominal viscera is obvious. Livingston has found that three points of attack in treatment by novocaine infiltration give benefit, and in some cases complete relief from pain and all associated phenomena. They are injection of the appropriate part of the sympathetic chain, of the peripheral nerves supplying the affected part, and of "trigger points" in the skin or deeper structures, which, exquisitely tender, seem to precipitate a new train of symptoms on the slightest touch. Sometimes one point of attack and sometimes another would give the required relief, the effective means varying according to individuals rather than to the particular type of case. The value of local infiltrations of novocaine in such cases has been noted by others, notably Leriche, but more significant is the fact that sometimes one, or several injections of novocaine repeated at intervals, will produce a permanent cure of a condition that has persisted and often progressed for many years. The cases are too numerous and too dramatic for one to ignore their significance in the physiology of pain. Livingston offers as an explanation the theory that the original lesion acts as a trigger point to set off a series of reflex changes in both the somatic and visceral (vasomotor, sudomotor, pilomotor) spheres, which gradually dominate the clinical picture and obscure the origin. The sympathetic pathways are prominently involved so that the pain is not confined to any single nerve nor to any segmental root distribution. The effect of these reflexes is to produce a state which accentuates the initial irritation, and a vicious circle is established. As the train of symptoms gathers momentum contralateral reflexes are brought into play and other cord segments become secondarily involved, so that the whole mechanism becomes increasingly difficult to stop. No doubt impulses from higher centers contribute to the disturbed physiological state which is produced in the cord. "Just as habit patterns and conditioned reflexes become fixed in time, so these disturbances may become so permanent that even removal of the original trigger point may not displace them. Hence in the treatment of pain syndromes it is important to attack them early before

permanent patterns are established. If the trigger point can be removed, its early elimination may lead to a subsiding of the abnormal activities and a return of normal function. If it cannot be found or removed, the repeated interruption of the various parts of the vicious circle may gradually destroy its momentum and permit normal physiological activities to regain ascendancy" (Livingston, personal communication). A similar mechanism must underlie the improvement following spinal anesthesia in many cases of megacolon.

Basing his ideas on the demonstration by Lewis & Pochin of a double pain response of the human skin to a single stimulus, Kendall (177) in a provocative discussion on "central pain" suggests that the "thalamic" quality of causalgia may be due to the functional dissociation of two sensory pathways. Interruption of the rapidly conducting fibers by a lesion may leave relatively intact the slowly conducting fibers which are normally inhibited by them. The greater the damage, the less is the likelihood of overreaction occurring. The hypothesis is a modification of views expressed by Head and Foerster, but while attractive, it is too simple in its present form to account for the vascular and other phenomena accompanying the pain in cases of causalgia, and it would not explain the causalgic pain in a phantom limb.

It may be concluded that the evidence to date is in full agreement with Hinsey's statement (151) that the impulses along visceral afferent fibers may produce responses in somatic as well as visceral effectors, just as somatic afferent impulses may elicit activity in visceral as well as somatic effectors.

VISCERAL EFFERENTS (AUTONOMIC)

Extremities.—Further work on the vasomotor nerves to the limbs, particularly in regard to the operative treatment of vasospastic states, has served to emphasize the necessity of a more exact anatomical knowledge of the pre- and postganglionic pathways. Recurrence of symptoms frequently follows sympathetic denervation of the arm for Raynaud's disease, and this is true whether the denervation be predominantly a postganglionic excision or a preganglionic section. The search for the cause of this relapse is one of the largest problems today in vascular surgery. Simmons & Sheehan (285, 286) found that, excluding cases of incomplete denervation, the relapses fall naturally into two groups.

The first group shows an early relapse and symptoms can be reproduced within a few days of operation. They are probably due to a local fault in the digital vessels which is sufficiently severe to nullify the effectiveness of the vasodilatation obtained by sympathetic denervation (Lewis). The second group of late relapses do not begin to show symptoms for several months after operation. They cannot be accounted for by increased sensitivity of the denervated blood vessels to circulating epinephrine, for this hypersensitivity is maximum eight to ten days after denervation and is perhaps less evident when clinical relapse becomes apparent. From skin-temperature studies following anesthetization of the ulnar and median nerves, Simmons & Sheehan have demonstrated that the relapse in these cases is accompanied by a regeneration of vasoconstrictor fibers. The rise in skin temperature following injection of the peripheral nerves is far greater than could be accounted for by the interruption of any slight discharge of impulses from decentralized ganglia.

The rapidity of anatomical regeneration and functional recovery within the autonomic nervous system has been well known since Langley first demonstrated its occurrence. Recently, in association with Phillips and Hare, Hinsey (155) has studied in cats the regeneration following pre- and postganglionic denervation of the extremities. After a true preganglionic sympathectomy of the forelimb (extradural section of thoracic ventral roots), preganglionic fibers regenerated in 36 to 61 days as detected by records of skin temperature and skin galvanic reflexes. Following removal of the sympathetic thoracic chain, from the stellate through T8, there was no evidence of regeneration to the pad vessels after nearly a year, but preganglionic fibers regenerated into the remaining cervical sympathetic trunk with return of sympathetic control of pupils, nictitating membrane, and ear vessels. In an important contribution to the study of degeneration and regeneration in sympathetic synapses, Gibson (109) finds, after section of the cervical sympathetic chain below the superior cervical ganglion, the first signs of regeneration in eleven days and reappearance of boutons forty-four days after operation, when restoration of function in the ganglion returned. Even complete removal of the entire sympathetic chain on both sides, according to the technique of Cannon, is not sufficient to prevent regeneration (127).

In man, regeneration of vasomotor fibers into the upper ex-

tr emity after division of the thoracic chain below the third ganglion presents no difficulty, but if removal of the stellate and second thoracic ganglia is a complete postganglionic excision for the arm, one must ask from whence come the new sympathetic fibers after cervicothoracic ganglionectomy. It is conceivable, though unlikely, that preganglionic fibers could extend down into the arm and take over the functions of the postganglionic neurons. Foerster (91) suggests that the middle cervical ganglion may be a source of postganglionic neurons to the upper extremity, and such fibers would be left behind in the usual cervico-thoracic ganglionectomy. A more likely explanation is offered by Livingston (213): that in man a considerable number of postganglionic cells related to the arm lie in ganglia below the second thoracic; the operation would then merely interrupt their axons and regeneration could be readily expected.

The sensitivity of denervated vessels to epinephrine, so convincingly demonstrated by White, Smithwick & Freeman, is well established in man. It is probably responsible for the drop in skin temperature in the hand seen a few days after any sympathetic denervation. It is greater after cervicothoracic ganglionectomy than after a predominantly preganglionic section, but the difference is not as marked as it appears to be in monkeys (82, 84). Lewis (199) has added new evidence that it cannot be the explanation of the failure of ganglionectomy to cure Raynaud's disease in the hands. If this were so, he argues, attacks of vasospasm should occur equally after sympathectomy, whether the cases operated upon were originally cases of Raynaud's disease or not. Such is not the case. Furthermore, in a series of six patients suffering from relatively early signs of Raynaud's disease, and examined shortly after a predominantly preganglionic section, Lewis found that the operation lowered the whole scale of abnormality of vascular tone without appreciably changing the relative positions of the members of the series, arranged previously according to the degree of severity of symptoms. He argues that such a result is to be expected if the effect of sympathectomy is the withdrawal of normal nerve impulses maintaining vasomotor tone, but not if the Raynaud's phenomenon is due to exaggerated autonomic activity, which if removed should reduce all cases to the same state. Lewis' contention that a "local fault" of the digital vessels underlies the vasospastic attack is strongly supported by such evidence. The "local

fault" may be obliterative vascular disease, or in mild cases "an increased susceptibility of the vessels to cold due to the sensitization of the vessels by some circulating hormone." The undue sweating, however, which is so frequently associated with attacks of vasospasm, and the occurrence of severe attacks of "dead white fingers" in certain patients on any emotional disturbance, point to an instability of the autonomic nervous system at least in certain individuals. It is likely that the digital vessels may become more susceptible than usual to various kinds of stimulation, to vasomotor impulses, to circulating epinephrine, or to direct cold, and there is probably considerable variation in the particular type of stimulation which is effective in individual cases. In other subjects, exaggerated autonomic activity may bring about the same condition as increased susceptibility, and both factors may operate simultaneously, each probably bringing about reflexes which enhance the other.

The anatomy of the pre- and postganglionic pathways to the limbs of the monkey has been studied by Sheehan & Marrazzi (282, 283) using the oscillographic method for recording activity in the various peripheral nerves after stimulation of ventral spinal roots. They found that the sympathetic preganglionic outflow from the spinal cord to the upper extremity extends from T4 to T8 (inclusive) with the major outflow from T5, T6, and T7, and to the lower extremity from T12 to L3 (inclusive) with the major contribution from L1, L2, and L3. These outflows are more restricted in origin than in the cat and dog. The existence of B as well as C potentials amongst the responses, shown to be autonomic by their disappearance after nicotine, implies the possibility of control over effectors differing in function and distinct from those supplied by fibers of the C group. Crossed pathways, between the sympathetic chains, of fibers destined for the lower extremities, were not demonstrated. The findings in the monkey cannot be transferred directly to man, but the fact that the outflows in cat, dog (71, 72), and monkey are so nearly the same, strongly suggests a similar arrangement in man. Smithwick (291), from his extensive clinical experience, believes that the second and third thoracic roots may sometimes contribute preganglionic fibers to the arm, and Kuntz, Alexander & Furculo (186) have advanced experimental evidence, as yet unconvincing, that the first thoracic nerve also plays a role in the sympathetic innervation of the upper ex-

tremity. In man, Foerster (92) has stimulated ventral spinal roots and made the following observations: stimulation of the ventral roots of T1 or T2 produced vasoconstriction of the ipsilateral face and neck, but no vasomotor changes in the arm; stimulation of the ventral roots of T3 to T6 (and sometimes T7) caused vasoconstriction in the upper extremity, as shown plethysmographically; stimulation of T8 and below had no vasomotor effect in the arm; vasoconstriction was evident in the lower extremity when the ventral roots of T10 to L2 (inclusive) were stimulated. The details of procedure in Foerster's experiments are not given, so it is not clear how body movements were eliminated, nor what variations in blood pressure occurred which might materially affect the interpretation of plethysmographic readings. However the close accordance of Foerster's findings in man with those in the monkey, cat, and dog are striking. It is highly unlikely therefore that the roots of T1 or T2 make any contribution to the sympathetic innervation of the upper extremity in man. The possibility that in man T3 may send vasomotor fibers to the arm remains open.

The location of the postganglionic cells related to the extremities is at present being investigated. In the arm, where the problem is one of more immediate clinical importance, surgeons have followed Langley's analysis in the cat, assigning the cells to the inferior cervical and first thoracic ganglia, and possibly extending down as far as the second thoracic. Livingston (209) advanced arguments in favor of still further extension downwards, to the ganglia of the third and fourth thoracic segments, and in 1932 recommended removal of the third thoracic ganglion alone, or the second and third, as preferable to the usual cervicothoracic ganglionectomy. This operation was first performed in June 1931 by Livingston. Subsequently Telford (1935) and Smithwick (1936) advocated a similar approach, but designing their operations with the intention of leaving postganglionic cells intact. Actually the retraction of the superior cut end of the thoracic sympathetic chain and its suture to the scalene muscle in these operations must produce considerable vascular disturbance in the ganglia, and the postganglionic neurons may thereby suffer more than is generally believed. An upward extension of postganglionic neurons related to the upper extremity is considered proven by Foerster (91). He stimulated in man the middle cervical sympathetic ganglion and obtained vasoconstriction in the upper extremity of the corre-

sponding side. This is supported by the experimental findings of Derom & Grimson (72). The middle cervical ganglion, in either its "high" or "low" type, (279) is invariably present and contributes rami to the fourth and fifth, sometimes the sixth, and more rarely the seventh, cervical nerves. In the lower extremity it seems likely that the synapses of the sympathetic nerve fibers to the feet are situated in the ganglia from the third lumbar to the third sacral. The third lumbar ganglion is included because it sends a grey ramus to the fourth lumbar nerve, which contributes sympathetic fibers via the femoral and saphenous nerves to the medial side of the foot (7). This can be readily demonstrated by the reflex sweating which persists in the distribution of the saphenous when the lumbar sympathectomy has been performed rather low leaving the rami to L4 intact. Recent studies of the peripheral pathways of the vascular nerves in the limbs (118, 181, 195) have confirmed the earlier accounts by Wingate Todd & Woollard.

There have been a number of clinical reports of advances in the medical and surgical treatment of peripheral vascular diseases, so this subject need not be discussed further. For further details Homans' important monograph (160) on the vascular diseases of the extremities should be consulted. Cornil and his associates (59) have again reported alterations of ganglion cells and thickening of the pericellular capsule and interstitial tissue in sympathetic ganglia in cases of vascular disease, and experimental arteritis has been produced in one femoral artery of the dog by the injection of a chemical irritant; two to four months later cellular and interstitial lesions have been found in the ipsilateral sympathetic chain only (60). Harrison (131) finds that experimental arterial disease induced by feeding rabbits on cholesterol is more severe in a sympathectomized limb than on the opposite normally innervated side. The reason is not clear. Though it is not possible to make direct comparisons between human and experimentally produced arterial disease it perhaps raises a question whether sympathectomy performed for Raynaud's disease may render the vessels more liable to subsequent atheroma. Sympathetic denervation has also led to an increase in the allergic and inflammatory reactions in rabbits (6, 20).

The methods of investigation of the limb before and after sympathectomy vary somewhat. The completeness of sympathetic denervation can always be ascertained by the absence of reflex

sweating in the part when the rest of the body is heated, or in response to faradism (21, 320), and by the absence of the pilomotor response to acetylcholine (56, 57, 270). It should be noted that prolonged heat applied directly to a completely sympathectomized part can produce a slight sweating even after peripheral nerve block with novocaine (286). This local effect may be due to chemical stimulation by dilator substances produced in the skin after exposure to heat

The plethysmograph, one of the earliest methods of recording vasomotor changes, is being increasingly used, with various refinements (45, 87, 111, 117, 167, 298). It has revealed that reflex peripheral vasoconstriction will occur in normally innervated extremities in response to many acute forms of stimulation, a sudden noise, pain, acute dilatation of the duodenum or esophagus, mental arithmetic, or even a deep breath. These reflexes do not occur in a sympathectomized area. Hertzman (143, 144, 145, 146), following a suggestion by Marrazzi (224) has designed a photoelectric plethysmograph for estimating the blood supply of various skin areas. It takes advantage of the fact that absorption of light by a transilluminated tissue varies with its blood content. The skin areas thus studied are arranged in descending order of the richness of their supply as follows: finger pad, ear lobe, toe pad, palm of hand, skin of forehead and face, dorsum of finger and hand, and of foot, forearm, knee, and front of leg. The fingers appear to be more rapidly affected by vasomotor reflexes than other skin areas and the skin of the forehead least of all; the toes, though slower to respond, show more prolonged effects than the fingers. Participation of the larger arteries in man in the response to normal vasoconstrictor stimuli is more irregular and less intense than that of the smaller vessels (145). Doupe, Newman & Wilkins (76) have shown that a rise in arterial pressure as measured in the fingers can occur coincidentally with a lowering of blood pressure measured proximally in the limb.

Grant & Pearson (117) found that body warming in mild degrees greatly increases the circulation in the hand and especially in the fingers, whereas it causes not more than a slight increase in the forearm, provided the circulation to the hand is arrested. Epinephrine in small doses likewise causes vasoconstriction in the fingers but an actual increase in the volume and blood flow in the forearm. Corresponding observations on the foot and leg reveal

the same difference between the proximal and distal parts. The findings have been extended to the separate parts of the finger (319). The large numbers of arteriovenous anastomoses in the digits and their absence from the forearm and leg undoubtedly play a part (326), but in addition the existence of sympathetic vasodilator fibers to the skin in the upper and lower extremities, particularly to the forearm and leg, play an important role. Grant & Holling (116) from studies on normal subjects have added further evidence to that already advanced by Lewis & Pickering (201), and Fatherree & Allen (83) in this regard. They showed that in contrast to the effect of mild warming, more rapid and intense heating of the body (excluding the limbs under consideration), causes a considerable increase in the volume of the forearm and leg, together with flushing and warming of the skin. This response is not present after sympathetic ganglionectomy. The inference is unmistakable that the vasodilatation provoked in the forearm and leg by heating the body is dependent upon the integrity of sympathetic vasodilator nerve fibers. An increase in paw volume upon injection of epinephrine in the cat, if the animal is kept cool, and irrespective of the effect of epinephrine on the blood pressure, has been shown by Goetz (112) to be due to an adrenergic rather than a cholinergic mechanism. He believes this dilator response to be due to a constrictor effect on the veins, resulting in venous congestion, passively increasing the paw volume. Such a mechanism however cannot account for the flushing and warming of the skin which accompanied the increase in volume in the forearm and leg in the experiments of Grant & Holling. The predominance of vasoconstrictor nerves in the hand and foot and of vasodilator fibers in the forearm and leg may explain why, following sympathectomy, there is a lasting improvement in the circulation in the hand and foot, and often none in the forearm and leg.

Measurements of skin temperature by Fatherree & Allen (84) have shown that in normally innervated extremities there are marked variations of the response to intravenous epinephrine in different digits of the same extremity under similar conditions and of the same digits on different occasions. The variations are much less after sympathectomy (82). Epinephrine usually produces a slower but more prolonged vasoconstrictor effect in the toes than in the fingers. There is insufficient knowledge of the normal fluctuations in skin temperature of various areas of the

body under controlled environmental temperature and humidity. Invaluable contributions to this subject have been made (218, 268, 269, 277) but further studies are needed. The skin of the extremities apparently plays a much more important role in the dissipation of heat than that of the head and trunk. There are probably significant differences in the response of the vessels in the upper and lower extremity to changes of posture which may account for some of the differences in the clinical result of sympathectomy in the two limbs. Lumbar sympathectomy has been shown to shift the regulatory control of the elimination of heat from the lower to the upper extremity (278).

Sudomotor fibers.—In an extensive study on sweat secretion in man List & Peet (208), using the technique of Minor (235), have investigated the distribution of sweating produced by various types of stimulation. Thermoregulatory and emotional sweating, though both centrally induced, are not identical in distribution. The latter is usually more localized to the palmar surfaces of the hands and fingers, the plantar surfaces of the foot and toes, the axilla, and the forehead. Sweating induced by pilocarpine and acetylcholine is most pronounced over the head, neck, and upper part of the chest. The gustatory reflex sweating (produced by eating spicy foods) is confined to the face, first on the upper lip and tip of the nose and later on the forehead and infraorbital areas.

It is well recognized that the sudomotor sympathetic fibers, in contrast to the vasomotor and pilomotor fibers (56, 57), are cholinergic (64) and that their distribution can be traced by mapping out the loss of thermoregulatory sweating following localized sympathetic removal or peripheral nerve block or section (125, 126). Hyperhidrosis of nervous origin can be effectively treated by sympathectomy (304, 317), but the area of anhidrosis so produced is accompanied by hyperhidrosis elsewhere, apparently a compensatory phenomenon, which disappears on the return of normal sweat secretion when regeneration of the sympathetic fibers occurs (136). Sympathetically denervated sweat glands, like smooth muscle, show a slight increased sensitivity to direct chemical stimulation, but the hypersensitiveness diminishes in time (208).

Pilocarpine and acetylcholine will induce some sweating even after sympathetic denervation. This is particularly true in the face. It was believed by Langley to indicate a direct action of such drugs on the sweat glands themselves. List & Peet however have shown

that, in man, sweating in response to pilocarpine and acetylcholine is abolished by section of a peripheral mixed nerve, if time is allowed for degeneration of nerve fibers. It persists however if the peripheral nerve is merely blocked by novocaine. They believe therefore that small doses of such drugs act on the nerve endings rather than directly on the sweat glands. Larger doses may excite the sweat glands directly. Since, however, degeneration of the sympathetic nerve endings to sweat glands does not abolish the sweating response to small doses of these drugs, there must be other cholinergic nerve endings in the distribution of the peripheral nerves, suggesting a double innervation of sweat glands.² Such cholinergic fibers of parasympathetic origin are well known in the cranial nerves but have not been demonstrated conclusively in the extremities.

Dorsal root potentials.—On the anatomical side Westbrook & Tower (316) have offered conclusive evidence that excision of dorsal root ganglia is followed two to four weeks later by growth of fibers (probably from injured ventral roots) into the central cut ends of the dorsal roots, and into the spinal cord. The rapidity of regeneration was not fully considered by previous workers, and unquestionably accounted for much of the anatomical evidence in favor of efferent fibers emerging in dorsal roots. Any truly persistent fibers in the central cut ends of dorsal roots might possibly be accounted for by occasional aberrant ganglion cells, as described by Duncan & Crocker (78) in the proximal stumps in the dog, but such cells are apparently much rarer in the cat (316). Convincing anatomical evidence of emerging fibers in dorsal spinal roots is therefore lacking. The most recent counts give a nearly one to one ratio in cells and fibers in dorsal roots and their ganglia (68, 158).

The conclusions to be drawn from the anatomical evidence in no way alter the fact that stimulation of the peripheral end of the cut dorsal root leads to peripheral vasodilatation, but whether such a mechanism plays any significant role in the vasomotor reactions of the intact animal is not yet known. Ascroft (4) observed the skin temperature changes in monkeys with one limb completely deafferented and found the reactions to variations in external temperature and to epinephrine were essentially the same on the two

² In twenty patients suffering from facial palsy Boot (24) found exaggerated thermoregulatory sweating, and believed that this indicated the existence of inhibitory sweating fibers in the trunk of the facial nerve.

sides. Full vasodilatation can still occur after deafferentation, and no sensitivity to epinephrine or acetylcholine is developed. Dole & Morison (75) have made simultaneous recording of the volume of a sympathectomized and of a completely denervated paw in cats and dogs, during reflex depressor stimulation, and found no differences between the reactions of the blood vessels of the two sides, although both increases and decreases of paw volume were produced. The evidence to date suggests that in the intact subject the vasodilator mechanism of the dorsal root fibers is purely a peripheral one, taking place probably by means of axon reflexes. However, the functional significance of the reflex discharges over dorsal spinal roots when various sensory nerves are stimulated, has still to be considered. During the past year Toennies (308) has shown that the reflex discharge is repetitive, that it can be evoked by physiologically selected afferent impulses, such as those set up by a patellar tendon, and that, although the size of the reflex is greatly augmented if the cord is cooled (13, 89), well-defined reflexes are regularly present when the cord is at a normal temperature. The results so far do not allow any final decision as to whether sensory impulses and the reflex discharge are carried in the same fibers or whether there is a special system of centrifugally conducting dorsal root fibers. As a result of recent work, Barron (12) no longer accepts the existence of recurrent collateral fibers of the type postulated in earlier experiments.

Striated muscle, bones, and joints.—The whole question of the influence of autonomic impulses in both the phasic and tonic contraction of striated muscle needs clarifying. One has to consider direct effects on the height and duration of contraction, and indirect effects brought about by alterations in blood flow. The latter may be brought about by vasomotor reflexes or by chemical substances produced by the striated muscle during contraction. Bülbring & Burn (38, 39), in studying the well-known effect of epinephrine in counteracting fatigue of skeletal muscle, observed an augmentation in the height of the contraction, despite a reduction in blood flow which would itself tend to diminish muscular tension. Similar augmentation is produced by ephedrine and cocaine. The effect of epinephrine must be mainly an improvement in the transmission of nerve impulses since it is much more pronounced when the stimulation is applied through the nerve than when it is applied to the muscle directly. According to Bülbring & Burn (40) sym-

pathetic influences or epinephrine can act in three ways on the contraction of skeletal muscle. They have a small action in augmenting the contraction of fully curarized muscle which must be due to an effect on the muscle itself. They have a more important action in improving neuromuscular transmission, probably by augmenting the number of impulses which become effective. A third action may be the improvement of the excitability and conductivity of the motor nerve fibers, though at present it is impossible to say how important this action is when there has been no interference with the natural circulation. The mechanisms by which these changes are brought about are difficult to understand, but they may be circulatory in nature, acting possibly on the blood vessels of the nerve trunks.

Vasomotor reflexes in skeletal muscle, of both constrictor and dilator nature, in response to epinephrine and carotid sinus impulses, have been recently studied by Grimson & Shen (121). Grant (115) found no essential difference between the hyperemia of exercise in a right arm long deprived by its sympathetic nerves and that in the left normal arm. The sympathetic nerves therefore would seem to play no essential part in provoking the local vasodilatation of exercise, which is in agreement with the view, first suggested by Gaskell, that the mechanism is one of release of vasodilator substance from the active muscle fibers. The movements of the ear (204) and of the oral vibrissae (205) in rabbits, described by Libelli, on stimulation of the cervical sympathetic trunk are probably due to a chemical mediator released on stimulation of vasomotor nerves.

Patients with an irritative peripheral nerve lesion, or with causalgia or Raynaud's disease, may exhibit, in association with the vasomotor phenomena, varying degrees of so-called "trophic" changes. The skin becomes shiny and tense, the nails horny and brittle, the joints swollen and stiff, and the bones show marked decalcification. Belief in true trophic nerves is no longer held, and the changes are now considered to be secondary to circulatory disturbances. Earlier workers had shown that the growth of the skeleton, deprived of the entire thoracolumbar autonomic nerve supply, is quite normal in young experimental animals, but Harris & McDonald (130) claimed that lumbar sympathectomy accelerated longitudinal growth in the paralyzed limbs of children who have suffered from anterior poliomyelitis, and Lexer (202) in an

experimental study of the healing of fractures in rabbits found some acceleration in callus formation following sympathectomy. Corbin & Hinsey (58) studied the radiological and histological appearance of bone in cats, following lumbar sympathectomy and dorsal root section. Their findings indicate that adult bone and joints maintain their normal structure and function for long periods of time when isolated from the sympathetic system. Asher & Dirr (6), however, report a diminished resistance to ultraviolet irradiation of bone and skin following sympathectomy in rabbits.

Intracranial structures.—The physiological regulation of the cerebral circulation has been recently reviewed by Forbes & Cobb (97, 98). The general consensus of opinion seems to be that parasympathetic fibers accompanying the facial nerve are the only nerves which cause active dilatation of cerebral vessels. The vagus, aortic, and carotid sinus nerves have no such effect. Activity of sympathetic fibers accompanying the internal carotid artery may result in a mild vasoconstrictor effect in the pial vessels, which according to Fog (93) and Lunn (216) is manifest only in vessels of larger diameter. Koopmans (183) finds cervical sympathetic stimulation sometimes leads to vasodilatation. Asenjo (5) makes the interesting observation that extirpation of the superior cervical sympathetic ganglion can alter in rabbits the electrical potentials from the cortex on the ipsilateral side. Busch (41) finds myelinated fibers (assumed to be sensory) only along the meningeal vessels; this may be correlated with the sensitivity of the dura to cutting and clamping, particularly in the neighborhood of large vessels. The pial blood vessels are accompanied by the usual nonmyelinated fiber network, containing occasional nerve cells.

Eye.—Kuntz, Alexander & Furculo (186) have confirmed the fact that stimulation of the ventral roots of T1 or T2 causes dilatation of the pupil and retraction of the nictitating membrane. In the cat and dog section of both these roots, or of their white rami, is necessary to produce Horner's syndrome. Stimulation of the ventral root of T3 causes retraction of the nictitating membrane only, although in the monkey dilatation of the pupil occurs (284). Fay (86) gives C8 as the root through which pupillary fibers leave the spinal cord, but a recent study (280) carried out in this laboratory of the individual variations in the thoracolumbar outflow of finely myelinated fibers makes this seem unlikely. The fibers to the eye emerging by the upper three thoracic nerves ascend in the cer-

vical sympathetic; the majority relay in synaptic connections in the superior cervical ganglion. Foley & DuBois (95), following their experimental analysis of the vagus nerve, have carried out similar studies on the cervical sympathetic trunk and have confirmed that it contains few if any afferent fibers. Stimulation of the cervical sympathetic may cause exophthalmos in certain animals but in man exophthalmos can develop in the presence of sympathetic paralysis (27, 257). The ocular manifestations of Graves' disease therefore are not necessarily produced by overactivity of the sympathetic innervation. Brunton (37) has found that acetylcholine produces proptosis in dogs even after removal of the superior cervical sympathetic ganglion, but rarely if a constant blood supply to the orbits is maintained, this fact suggesting that the main action of acetylcholine in the intact animal is a relaxation of blood vessels, which, when distended with blood, push the eyeball forward. The role of inhibitory impulses in the pupillary dilatation of the light reflex (293) and in the response to pain (311) have again been studied (275). Accommodation of the eye for near and far vision involves reciprocal action of the sympathetic and parasympathetic (oculomotor) nerves (239). Ury & Oldberg (312) have presented a general scheme of the factors influencing pupillary diameter, and have used the dilatation of the pain reaction as an indicator in their study of the cortical representation of pain. The afferent pathways in the spinal cord for reflexes of the nictitating membrane, like those described by McSwiney for pupillodilator reflexes, lie mainly in the lateral columns of the cord, and are both crossed and uncrossed (3). A comparative and anatomical study of the ciliary ganglion has been contributed by Evans & Minckler (81).

Pituitary and thyroid.—Hair's (128) investigation of the nerve supply of the hypophysis has confirmed many earlier observations and has added a possible parasympathetic contribution from the carotid plexus, since bilateral superior cervical ganglionectomy leaves intact many unmyelinated fibers along the blood vessels in the pars distalis. There are however other possible sources of sympathetic fibers, more particularly along the vertebral vessels.

The morphological changes in the supraoptic nucleus and hypophysis and the physiological effects of section of the hypophyseal stalk in man resemble closely experimental findings in the monkey (260). The existence of a supraoptic hypophyseal tract in all animals and in man is established beyond any doubt (137, 220, 259).

Its role in the regulation of water exchange has been confirmed by Biggart & Alexander (22). Magoun, Fisher & Ranson (219) have reported that in the monkey, transection of the infundibular stalk through the median eminence results in a marked polyuria, while transection below it is compatible with the maintenance of a normal urine output. Mahoney & Sheehan (221), who did not obtain polyuria in monkeys, purposely placed the transection well below the median eminence; however, Keller (174, 175) has separated the hypophysis from the hypothalamus above this level without causing polyuria. Ranson and his colleagues interpret their results as follows. The neurohypophysis is believed to elaborate an antidiuretic factor at a rate controlled by the hypothalamus through the supraopticohypophyseal tract (165). In the experiments where the median eminence was left proximal to the transection, and still in connection with the hypothalamus, it might furnish sufficient antidiuretic hormone to maintain a normal water balance. Where the transection passes through or above the median eminence all source of antidiuretic substance is presumably removed, and diuretic factors holding sway, polyuria results. Keller's experiments however suggest that a source of antidiuretic hormone, other than the neurohypophysis, must be considered, possibly the hypothalamus. The diuretic factors are not yet fully understood. That they are under the control of the adenohypophysis seems established, but they are not necessarily secreted by it, for the adenohypophysis can be entirely removed in such experiments without affecting the polyuria (173). Denervation of the kidney likewise has no effect (15). The role of adrenal cortical hormone, in association with the hypophysis, in the metabolism of sodium and chloride has recently been investigated (322). For further details "The Hypothalamus" (*Research Publications of the Association for Research in Nervous and Mental Diseases*, 20, 1940) should be consulted.

The glycosuria resulting from stimulation of the superior cervical sympathetic ganglion, presumed to be due to a liberation of a pituitary hormone, could not be obtained by Hill & Maycock (150) in a series of carefully controlled experiments.

A vasomotor innervation of the thyroid gland is well known (Nonidez, 1935) but there is at present no direct proof of an autonomic secretory innervation (300). Uotila (310) from histological studies of the thyroid gland in the rat finds that bilateral

cervical sympathectomy causes a temporary and mild hypoactivity of the thyroid, but believes the effect is mediated through the anterior pituitary, rather than being a direct one. A similar mechanism is tentatively put forward by Friedgood & Cannon (102) to explain the signs of hyperthyroidism (increased basal metabolic rate, tachycardia, and unilateral exophthalmos) which they were able to produce in two cats (out of twenty-eight) with an end-to-end anastomosis between the proximal end of the phrenic and the (upper) distal segment of the divided cervical sympathetic trunk. Resection of the anastomosis in one animal brought the metabolic rate down to the normal level. The results confirmed similar interesting findings by Cannon in 1915.

Heart and lungs.—Continuing his studies of the innervation of the heart, Nonidez (245), using the choral hydrate formula of Cajal's method of staining, has found that in young animals the afferent, preganglionic, and parasympathetic postganglionic fibers stain deeply, whereas those which remain faintly stained are sympathetic postganglionic. Whether such differences are also found in the nerves of the adult has not yet been settled. If this differential staining holds true in other regions, it would lead to far-reaching developments in the unravelling of the autonomic pathways. It apparently bears no relation to the diameter of the fibers, but whether the weak staining of the sympathetic cells and fibers is an indication of their late development or whether it is in some way related to the chemical substratum of the neuron is still undecided. If it depends on chemical factors, it would seem more likely to differentiate adrenergic from cholinergic fibers, rather than sympathetic from parasympathetic, which is after all purely an anatomical grouping. It is of course true that the preganglionic and the majority of the parasympathetic postganglionic fibers are cholinergic, whereas the sympathetic postganglionic fibers are for the most part adrenergic, but in the heart, as elsewhere, there is considerable admixture. The existence of efferent cardioaccelerator fibers in the vagus, for example, has been confirmed (32), and Kabat (170) has shown that they are chiefly to be found in the right vagus and are probably adrenergic in type. Similarly cardio-inhibitory elements have been found in the sympathetic innervation of the heart (140).

Nonidez (245) has given a detailed account of the origin and components of the cardiac nerves in the dog. There is an important

exchange of fibers between the middle cardiosympathetic nerve and the vagus. The parasympathetic postganglionic fibers end chiefly on the structures above the coronary sulcus, few deeply stained fibers being seen in the ventricles, which are abundantly supplied by the sympathetic. The sinoatrial and atrioventricular nodes are particularly rich in parasympathetic nerve endings but, even after section of the atrioventricular bundle, stimulation of the left vagus will slow the rate of the dog's ventricle (169). There is no evidence in the heart, according to Nonidez, for a common terminal syncytium between the sympathetic and parasympathetic innervations, as suggested by Stöhr (295, 296, 297). Other anatomical investigations of the cardiac nerves have included dissections in the monkey (25), and histological studies of the intracardiac ganglia (46) and of the pericardium (240). Chamberlain (49) found that, in man, resection of the cervical sympathetic ganglia has no effect on the form of the electrocardiogram. Stimulation of the right cervical sympathetic may change the form conspicuously, decreasing the R or increasing the T waves, or both, but stimulation of the left sympathetic produces no constant changes.

The studies of Nonidez confirm Woollard's account of the innervation of the coronary vessels, the parasympathetic supply being particularly evident in the proximal parts of the vessels. The evidence of Katz & Jochim (171)—that in the dog the vagi carry only cholinergic coronary vasodilator fibers, whereas the stellate sends adrenergic, both dilator and constrictor fibers, with the latter predominating—would bring the coronary vessels into line with the rest of the vascular tree in regard to autonomic innervation. If this is confirmed, then paravertebral block of the upper thoracic sympathetic chain or stellate ganglionectomy may not only interrupt afferent pain pathways as previously supposed but also efferent vasoconstrictor fibers (213).

The peripheral innervation of the supracardial bodies (aortic paraganglia) in the cat has been studied by Hollinshead (157). The nerve supply appears to be entirely sensory, and to be derived chiefly from the left vagus nerve. The role of the supracardial bodies as chemoreceptors, such as has been advanced for the carotid body, has been demonstrated (55).

Daly, Foggie & Hebb (65), from experiments on the perfused lung preparation of the dog, conclude that large doses of epinephrine cause constriction of all parts of the pulmonary vascular

bed, including the capillaries, and that small doses cause arterial and venous constriction only, the effects being suppressed or reversed by ergotoxine. The significance of an occasional preliminary vasodilatation in response to the first dose of epinephrine is discussed by Petrovskaja (252). Large doses of acetylcholine also constrict the pulmonary blood vessels of the dog, an effect which is potentiated by physostigmin and suppressed by atropine, but in contradiction to previous observations, Foggie (94) finds that epinephrine also tends to suppress or reverse the constrictor effect of large doses of acetylcholine. Further work is necessary before the discrepancy between the results can be interpreted.

General vasomotor activity.—In the course of work on the innervation of the kidney, Homer Smith and his associates (290) have recently added evidence which has a bearing on vasomotor control elsewhere in the body. Normal human subjects subjected to high spinal anesthesia, up to T5 and higher, with no surgical intervention, showed no significant reduction in diastolic blood pressure nor change in renal circulation. It would seem therefore that the arteriolar bed generally, apart from the skin, must possess considerably more autonomy than has generally been supposed, sufficient in fact in the normal individual at rest in the supine position to maintain an essentially normal blood pressure. On tilting the normal person from the horizontal position, however, reflex vasoconstriction comes into play serving to maintain the arterial pressure at normal levels. This reflex response to change in posture was absent in the subjects after high spinal anesthesia, this fact showing that in these experiments the vasomotor fibers in the ventral roots were fully anesthetized. Smith (289) stresses the fact that many observations supporting the conception of a generalized vasomotor activity of the autonomic nerves have been made on anesthetized animals and that it has been shown that anesthesia itself leads to an increase in autonomic activity. It remains to be determined to what extent autonomic activity may be evoked by traumatic excitation, by excitement, and particularly by assumption of the upright posture. It seems likely that the autonomic nervous system will be found to play a significant role in the vascular changes necessitated by such activities of normal life.

Evidence in support of Smith's contention is afforded by the experiments of Hermann and his associates (142) who have destroyed the spinal cord of dogs below the level of T1 and have

found that the animals have been able to maintain a blood pressure within normal limits. During the last year they have shown that section of the splanchnics and vagi, and bilateral excision of the stellate ganglia do not alter the blood pressure under these conditions. The body temperature was also maintained. Excellent reviews of the regulation of peripheral vasomotor tonus have been contributed by Hermann (139) and Malméjac (223). There seems to be ample evidence to show that the sympathectomized animal has a resting blood pressure little different from the normal, but vascular responses can be elicited in these animals. Bacq, Bremer, Brouha & Heymans (9) have confirmed their original findings that occlusion of the carotid arteries in the totally sympathectomized cat causes a reflex arterial hypertension, but in the absence of the carotid sinuses the most predominant vascular response of the sympathectomized animal to any stimulus is a fall in blood pressure. Thus, Brown & Maycock (36) have shown depressor responses in the sympathectomized cat to brief occlusion of the vertebral arteries, sensory nerve stimulation, and stimulation of ventral spinal roots. The responses are abolished by curare, strongly suggesting that they are attributable to the vasodilatation consequent on muscular movement and not to a vasodilator mechanism in the dorsal spinal roots. However, irritation or section of the spinal cord may cause a fall of blood pressure in the sympathectomized cat, even after section of the vagi and full curarization, indicating possibly the interruption of a central vasoconstrictor tone exerted through some "extrasympathetic" pathway (36). These conclusions are substantially in agreement with those of Bacq and his associates (9).

In the normal animal alterations in blood pressure will bring about changes in the activity of the autonomic nervous system, obviously designed to maintain the internal equilibrium. The carotid sinus and aortic reflexes are perhaps the best known, but even after denervation of the carotid sinuses and bilateral vagotomy, Gellhorn, Darrow & Yesinick (108) found that a rise in blood pressure produces parasympathetic excitation and sympathetic inhibition, whereas a fall in blood pressure has the opposite results. The effects of variations of blood pressure extend to the activity of the somatic nervous system (107).

Hypertension has been produced experimentally by a variety of methods. Partial and sustained occlusion of the renal arteries

in dogs, as carried out by Goldblatt, leads to a form of hypertension which is not modified by various types of sympathectomy, though decapsulation of the ischemic kidney and promoting the development of a collateral circulation may cure the condition (47). In experimental hypertension in rabbits produced by constriction of the renal artery there is a hypersensitivity to pitressin and an abnormal pressor response to noise and fright (248), indicating a generalized increase in the reactivity of the muscular coat of the arteries, as was first suggested by Heymans (148). A second method of producing experimental hypertension is that described by Heymans & Bouckaert (149) and recently confirmed by Grimson (120), but not by Goldblatt and his associates (113), of section of the carotid sinus and aortic depressor nerves. Here again sympathectomy fails to alter the blood pressure significantly (120). A third type of experimental hypertension, which follows increased intracranial pressure, however, was abolished by total sympathectomy; if, however, the upper thoracic sympathetic ganglia on one side are left intact the elevation of blood pressure persists (101). Interpretation of these findings must await a clearer understanding of the mechanisms by which these various forms of hypertension are produced. The relative share that increased minute output, alterations in aortic capacity and elasticity, and total peripheral resistance play in such pressor effects has been timely stressed by Wiggers and his associates (74). Furthermore in these experiments, and in the surgical treatment of essential hypertension in man, where favorable results continue to be reported (62, 63, 249, 292) particularly in the relief of subjective complaints in selected cases, the rapidity of regeneration even after extensive removal of the sympathetic chain must be borne in mind in any evaluation of long-term results.

Adrenal medulla.—Experimental degeneration studies by Young (325), and physiological evidence advanced by Maycock & Heslop (230) on the innervation of the adrenal glands support the earlier findings of Hollinshead and of Swinyard, cited by Hinsey (151). The sympathetic fibers for the adrenal medulla arise from the spinal cord in the lower six thoracic and upper three lumbar spinal roots. No evidence of a bilateral innervation was found. Elliott's contention that the fibers are preganglionic has been confirmed. Total denervation of the gland can be assured by cutting all the splanchnics and removing the upper three lumbar sympathetic

ganglia. Hasama's (134) belief in a vagal innervation of the adrenals receives no support.

Spleen.—Hermann and his associates (142) have destroyed the spinal cord below T1 in dogs; the preganglionic fibers subsequently degenerate, leaving the splanchnic nerves composed only of post-ganglionic and afferent fibers. Stimulation of the distal end of the divided splanchnic nerve still produces a contraction in the volume of the spleen, thereby demonstrating that the synapses on the vasoconstrictor pathways to the spleen lie in the sympathetic chain. This is not so in the case of the kidney.

Liver.—In the perfused liver of the dog epinephrine may produce three types of vascular response (48, 172). It increases the venous outflow (by opening the hepatic veins), thereby diminishing the liver volume. It increases the resistance on the inflow side due to constriction of the small vessels of the hepatic artery and portal vein, and so again diminishes the flow through the liver. With the liver volume already small, it may increase the resistance on the outflow side with a resulting increase in liver volume. The possibility of obtaining these different responses explains the diversity in findings of previous investigators. Chakravarti & Tripod (48) found that, when observations were made early in the perfusion and when epinephrine is present in the blood, acetylcholine injected into the hepatic artery causes a dilatation of the hepatic artery, leading to expansion of the liver volume and diminished portal flow. The latter they interpret as being due to the swelling of the liver lobules. Katz & Robdard (172) believe that it is accentuated by a closure of the hepatic veins, a reverse of the effect of epinephrine. However, acetylcholine has no effect when injected into the portal vein (48). It is difficult to understand why an action on the efferent veins should only occur when the drug is injected into the hepatic artery. The observations serve to indicate the complexity of vascular responses to autonomic activity.

The association of gall bladder disease with abnormal function of the intestine, particularly the colon, has long been recognized. Goldman & Ivy (114) have recently shown that distension of the colon or stimulation of the proximal end of its divided nerve supply causes an inhibition in the flow of bile from the liver. Section of the nerves surrounding the hepatic artery abolishes this reflex. If such a stasis in bile flow were continued by repeated stimuli from the colon, precipitation of calculi might be favored. Whether the reflex

is due directly to inhibitory secretory fibers or indirectly to vascular changes has not yet been decided.

Pancreas.—Since the observations on the effect of splanchnic nerve stimulation on pancreatic secretion by Babkin and his associates, reported by Rosenblueth (265), Sergeyeva (274) has added the observation that clearly recognizable changes occur in the islands of Langerhans, the α -cells being involved on stimulation of the splanchnic nerves, and the β -cells when the abdominal sympathetic had been removed. Some of the changes suggest the interesting possibility that acinous tissue in the pancreas may under certain conditions be transformed into endocrine tissue.

Gastrointestinal tract.—Special dissections of the efferent innervation of the esophagus in man have been made (61, 236). Mitchell (236) directed his attention particularly to the gastroesophageal junction in view of Knight's suggestion of excision of the left gastric artery for relief of cardiospasm. Two reasons for the failure of this operation become obvious, firstly that the sympathetic nerve supply to the gastroesophageal junction in man comes from several sources, so that removal of the left gastric artery cannot produce the intended complete sympathetic denervation, and secondly that parasympathetic fibers may also be cut. Cardiospasm in dogs has been reproduced experimentally by bilateral vagotomy combined with an encircling incision through the outer coats of the esophagus just above the diaphragm (122). Since neither procedure alone is sufficient to produce the condition it may be concluded that there are fibers coursing within the wall of the esophagus which are related in some way to relaxation of the cardiac sphincter. Telford & Simmons (304) have reported good results in one case of cardiospasm after spinal anesthesia, thereby bringing the treatment in line with that of megacolon, which has also been attributed by Hurst to a failure of relaxation of sphincters.

Nothing new has appeared in regard to the peripheral innervation of the stomach. Edwards & Baker (80) have reported the statistical variations in the formation of the splanchnic nerves in man. The action of drugs on the intestinal wall, sensitized by division and degeneration of the fibers from the vagi and splanchnics, leads to the broad generalization (77) that vagal fibers are preganglionic, and that those of the mesenteric nerves are postganglionic, the synapses being presumably in the celiac and superior mesenteric plexuses. The inner layer of circular muscle of the intes-

tine contains a rich network of "interstitial cells of Cajal," which are considered by Li (203) as primitive nerve cells and as important constituents of the enteric nervous system, but this is a much disputed point. A new study of the influence of the extrinsic nerves and various drugs on the secretion by the small intestine has been made (324). Vagal stimulation (or splanchnic section) in decerebrate cats causes a secretion from the duodenum (Brunner's glands) but not from the jejunum or ileum. Cutting all the pre-ganglionic sympathetic fibers to the intestine or the injection of physostigmin subcutaneously causes secretion from all parts of the small intestine, and the addition of acetylcholine may cause severe damage to the intestinal mucosa. Presumably in the intact animal the gut produces a relatively large quantity of acetylcholine, which is prevented from acting by the inhibitory influence of fibers within the sympathetic innervation. The inhibition can be overcome experimentally by the administration of physostigmin or by section of the appropriate sympathetic nerves (324). Such an influence of sympathetic fibers in the secretory activity of the intestine raises the question of a similar mechanism in regard to its motility and suggests a possible treatment in acute intestinal obstruction. Resection of the celiac ganglion however appears to have no effect on the survival time of cats with intestinal obstruction by experimentally produced ligation (88).

Kolossov & Mechteriakov (182) in a study of the innervation of the rectum found degenerating fibers not only in the external but also in the internal sphincter 72 to 120 hours after section of the pudendal nerves, and believed them to be sympathetic fibers in the pudendals. It seems likely that they were vascular fibers, in which case a pathway along a mixed peripheral nerve would be entirely in keeping with what is known of vascular nerves elsewhere in the body. In a discussion on megacolon Telford (303) recommends division of the lumbar splanchnics on both sides, together with removal of the lumbar sympathetic chain. Takáts (302) suggests injection of small doses of acetylcholine to test the condition of the musculature before operation is undertaken. Telford, and others (54), have obtained remarkably good results from treatment by spinal anesthesia as recommended by Merle Scott and his associates (294). One possible explanation of this therapeutic effect of spinal anesthesia in megacolon is that there is an imbalance between the inhibitory and excitatory nerve impulses,

which is upset by anesthesia and a normal rhythm is re-established. Hurst (161) for example reports degenerative changes in the myenteric plexus in certain cases of megacolon and believes the condition may arise from some disturbance in the nervous control of the sphincters, resulting in a failure of their relaxation. Ross (267), however, suggests the interesting possibility that in certain individuals there may be developmentally an anatomical gap between the end of the vagal and the beginning of the sacral innervation to the large intestine, and that in this interval the sympathetic nerves (presumably mainly inhibitory) provide the only extrinsic innervation. This may operate in certain cases; it at least opens up again the unsolved problem of exactly where the vagal supply to the gut ends.

Reproductive organs.—Reynolds (262) in a recent monograph has reviewed the anatomical and physiological literature dealing with the uterus. Since Langley it has been customary to regard the uterus, at least in the cat, as innervated by sympathetic nerve fibers only, but physiological studies have now established beyond question the presence of a parasympathetic (sacral) outflow. Sheehan & Labate (in unpublished observations) have noted increased activity in the uterus and tube in the monkey on stimulation of the ventral roots of S1, S2, and S3. Gross anatomical preparations (237, 272) confirm the existence of a sacral outflow to the uterus and tubes. The finer anatomy of the nerve terminations in the ovary (110) and the testis (315) have also been restudied during the past year. Labate, in this laboratory, has recently made a special investigation of the effects of nerve stimulation and of the administration of various drugs on the activity of the uterus and tube. The results vary according to species, to the phase in the estrus cycle (69), and to the presence or absence of pregnancy. Morison (241) led off action potentials from uterine muscle and observed that the changes brought about by epinephrine or by nerve stimulation are apparently confined to that part of the muscle to which the stimuli are delivered. This would indicate a limitation of the concept of uterine muscle acting as a syncytium. Administration of estrin to ovariectomized rabbits causes hyperemia of the uterus, but the effect is abolished by atropine, suggesting the possibility that acetylcholine may be the intermediary factor. Reynolds & Foster (263) have found that the acetylcholine content of the uterus is greatly increased by the administration of selected estro-

gens into ovariectomized rabbits. The clinical results of excision of the hypogastric plexus (presacral nerve) for primary dysmenorrhea have been reviewed by Meigs (231).

Bladder.—The most important work to be published on bladder innervation during the past year is the monograph by Langworthy and his associates (192). They review the literature completely in the light of their own researches in the experimental laboratory and in the hospital, of which only the briefest summary is possible here. The sympathetic nerves are distributed to the blood vessels and to the trigone, to Bell's muscles and the crista urethrae; there is no evidence that they innervate the muscle (longitudinal or circular) of the bladder wall or urethra, which is supplied exclusively by the sacral parasympathetic outflow (191, 193). Afferent fibers run in both sympathetic and parasympathetic pathways. Micturition begins with a contraction of the vesical muscle and only after the intravesical pressure rises does the "internal sphincter" open.³ The external sphincter opens later, but it apparently cannot be opened voluntarily. The only voluntary power lying in the external sphincter is the ability to contract, to postpone or stop micturition.

Stimulation of the sacral roots which contain the parasympathetic fibers will produce contraction and emptying of the bladder; the contraction can be seen unilaterally if the nerves are excited on one side. Section of the parasympathetic fibers leads at first to an inability to empty the bladder, so that the walls become stretched and urine escapes only when the pressure becomes great enough to force the urethra open mechanically. When a longer time has elapsed the bladder will often empty automatically. Injuries of the cauda equina in man, if complete, are comparable to such a section of the sacral roots in the experimental preparation. The patient remains aware of pain with overdistension of the bladder inasmuch as the sympathetic afferent pathways are intact.

When the sympathetic supply is stimulated, the ureteral orifices close and are pulled towards the midline; the base of the bladder moves downwards, carrying a portion of the mucosa towards the vesical orifice. The movement is slow and of small amplitude, not persisting if the stimulating current is continued for

³ The musculature surrounding the vesical orifice and posterior urethra is a continuation of the detrusor muscle. There is no antagonism between the detrusor and the muscles around the vesical orifice except as it is relative to the anatomical arrangement of the fibers.

several seconds. There is also, in the male, contraction of the prostatic musculature and of the smooth muscle of the seminal vesicles and ejaculatory ducts. Section of the sympathetic nerves produces no appreciable modification of vesical activity. The closure of the "internal sphincter," seen by Learmonth (196) on stimulating the hypogastric plexus in man, has been interpreted as supporting the widely accepted theory that the sympathetic fibers inhibit the vesical muscle and contract the "sphincters." Any action of the sympathetic fibers upon urethral resistance, however, acts, according to Langworthy *et al.* (192), not at the vesical orifice but in the prostatic urethra, and has only a sexual function. The sympathetic fibers, he believes, have solely vasomotor and sexual functions in relation to the bladder and urethra. Langworthy's theory discards any antagonism between the sympathetic and parasympathetic innervations of the bladder, and it throws a new light on the bladder symptoms in man, which have been erroneously referred to as "sphincter disturbances."

TRANSMISSION AT THE SYNAPSE

The microscopical appearances of the sympathetic synapse in normal, degenerating, and regenerating phases, have been studied by Gibson (109) with the silver technique of staining. *Boutons terminaux* and *boutons de passage* can be seen, similar to those existing in the central nervous system but their number and size are surprisingly small. The largest number of boutons of whatever type seen on one cell and its processes was thirteen; compare this with the hundreds counted on one ventral horn cell in the spinal cord. The difference may be due to difficulties in staining technique but it must be remembered that there are other synaptic structures (pericellular nests, etc.) within sympathetic ganglia (184, 185). Degenerative changes in the boutons within the superior cervical ganglion were traced by Gibson (109) from the second day after division of the cervical sympathetic trunk, but the ganglion cells themselves were not affected by degeneration of the preganglionic fibers. Regeneration of boutons was first seen forty-four days after operation, and the coincidental functional recovery in the ganglion was followed closely by electrophysiological recording. The degeneration studies of Gibson and Kuntz support the theory of structural discontinuity in the sympathetic nervous system, in opposition to Stöhr (295, 296, 297) who rejects the neuron doctrine as

applied to the autonomic nervous system and claims that there is no interruption of the fiber pathway at the synapse. The syncytial conception of Stöhr cannot be supported by any appeal to more primitive types of nervous systems, where discontinuous neurons are the rule, according to Woollard & Harpman (323). In view of this, the existence in higher animals of a neurencytium amidst an otherwise synaptic system would be surprising. Furthermore the absence of any significant changes in the end bulbs on ventral horn cells following peripheral nerve section, as reported by Barr (11), lends no support to the claim of neurofibrillar continuity at the synapse.

For a detailed account of the present views regarding transmission of nerve impulses at the synapse, the reader is referred to recent reviews by Bronk (28), Brown (34), Eccles (79), Forbes (96), Lorente de Nó (215), Newman (244), and Rosenblueth (265). The release of chemical substances (sympathin and acetylcholine) in visceral tissues at the neuromuscular and neuroglandular junctions is well proven. Gaddum & Kwiatkowski (105) strengthened their previously reported evidence that the substance liberated in a perfused ear of the rabbit on stimulation of postganglionic sympathetic fibers resembles epinephrine rather than any of its allied compounds. The properties of the substance so released have been reviewed by Hermann and his associates (141). Lambert & Rosenthal (188) believe that there is also a release of a histamine-like substance in the skin of the rabbit's ear on stimulation of the cervical sympathetic trunk. Lissák (207) has added further proof that acetylcholine and sympathin are contained in and liberated directly from nerve fibers, the former from preganglionic and from postganglionic cholinergic fibers, the latter from postganglionic adrenergic fibers. The mere presence of acetylcholine or even an increase in its output during nerve excitation is not suggested as a proof that it is actually concerned in the transmission. A similar study fundamentally in agreement with these results has been made by Chang and his associates (50). Acetylcholine has been extracted from the placenta, and, since this structure is supposed to be without nerve fibers, the source of the acetylcholine may easily have been the pregnant uterus from which acetylcholine has also been extracted (263). Loewi & Hellauer (214) found that the acetylcholine content of sympathetic preganglionic fibers is six times greater than that of sympathetic postganglionic (adren-

ergic) fibers. The possibility that myelination might account for some of this difference led to estimations on predominantly myelinated somatic nerves, such as the optic nerve and dorsal spinal roots, both of which proved to be free of acetylcholine. Loewi believes that this fact argues against the production of acetylcholine at the central synaptic endings of such fibers, i.e., within the central nervous system.

With regard to the electrical phenomena within autonomic ganglia during transmission of nerve impulses, Therman, Forbes & Galambos (305), in repeating earlier experiments of Eccles, have used microelectrodes to record the activity from the surface of the superior cervical ganglion. The axon-like spikes obtained during preganglionic stimulation possessed characteristics which strongly suggest that they are derived from single cells.

Further studies by Cannon & Rosenblueth (44, 266) of the stages in the response of striated muscle to repetitive stimulation of the motor nerve at high frequency lend support to the chemical theory of neuromuscular transmission. The work on the chemical changes within autonomic ganglia during the past year has centered particularly around alterations of potassium and calcium. It is well known that, if during perfusion of a ganglion with acetylcholine, the calcium concentration is increased or the potassium decreased, the frequency of impulses discharged by the acetylcholine-activated cell is greatly reduced; and conversely an increase in the concentration of potassium ions or a reduction in calcium augments the rate of activity developed by a given amount of acetylcholine (28). Calcium ions, however, are essential for the transmission, both at the neuromuscular junction (35) and at the ganglionic synapse (30). Harvey & MacIntosh (133) have shown that if the superior cervical ganglion is perfused with a calcium-free solution, there is no release of acetylcholine from the preganglionic nerve endings on stimulation of the sympathetic trunk. The associated abolition of synaptic transmission cannot be due to a loss in power of conduction of the ganglion cells, for, in the absence of calcium, they exhibit a long-continued spontaneous activity, in the form of a repetitive discharge of impulses along the postganglionic axons. The preganglionic axons also appear to be abnormally excitable. Harvey & MacIntosh (133) therefore postulate that, in the absence of calcium, potassium ions fail to produce their normal liberation of acetylcholine, which they regard as the

synaptic transmitter. In the light of these experiments Shafer's assumption (276) that calcium ions are only necessary for functioning at parasympathetic synapses cannot be accepted. Comparable results to those of Harvey & MacIntosh have recently been obtained on brain slices *in vitro* where the usual liberation of acetylcholine by potassium failed in the absence of calcium ions. The extraction of acetylcholine from the brain has been confirmed by two independent investigations (52, 301). The results have been interpreted as supporting indirectly the view that acetylcholine acts as a synaptic transmitter in the central nervous system. Changes in the electrocorticogram in the cat and rabbit, induced by the local application of minimal amounts of physostigmin and acetylcholine, indicate cortical stimulation and facilitation of the cortical synapses (233), yet, if Kuo's (187) observations can be confirmed, there is apparently in the developing chick no time relation between the first formation of acetylcholine and the appearance of reflexes through the nervous system. Physostigmin and other anticholinesterases curiously augment some spinal reflexes, while depressing others (232). A modification of the acetylcholine concentration at specific regions of the grey matter would explain such variable central actions, according to Schweitzer, Stedman & Wright (273).

That epinephrine exercises a specific inhibitory action on sympathetic synapses has been demonstrated by Marrazzi (225, 226, 227) by recording the decrease in action potentials in the postganglionic nerves of the superior cervical ganglion when epinephrine is either liberated from the adrenals by splanchnic stimulation or is injected intravenously in amounts comparable to those known to be secreted under stress. Ephedrine injected intravenously exercises a similar though less marked effect. Marrazzi (228) suggests an adrenergic inhibitory mechanism in synaptic transmission, and points out that the ganglionic inhibitory action of epinephrine may constitute a self-limiting mechanism capable of checking the widespread activity produced by sympathicoadrenal discharge when this has reached a high level. Such a mechanism may explain the diminished reflex excitability of the autonomic nervous system on the injection of epinephrine reported by Darrow & Gellhorn (66). Marrazzi (226) has also found by recording the postganglionic action potentials that atropine depresses and pilocarpine potentiates transmission in the superior cervical ganglion;

and points out that the similarity of these ganglionic actions to those at peripheral junctions makes it misleading to speak of "nicotine-like" as distinct from "muscarine-like" effects. Bender & Weinstein (19) have suggested that an excess of epinephrine may stimulate the liberation of a cholinergic-like substance in the body. Conjugation, rather than oxidation, seems to be the main physiological method by which epinephrine is inactivated in the body, according to Richter (264).

The epinephrine-like action of various anesthetics has been studied by Tripod (309), MacGregor (217), and Philpot (253), and further investigations have been carried out on the action of ergotoxine in producing a reversal of the effects of stimulating sympathetic nerves in certain organs (1, 123, 206). Bussell (42) has found that atropine, generally regarded as antagonistic to acetylcholine, may also have an antagonism to epinephrine, but he appears to have overlooked the demonstrated action of atropine at the ganglion (226). Atropine is related chemically to cocaine which also depresses the action of acetylcholine and, in large doses, that of epinephrine. Gellhorn & Darrow (67, 106) have continued their studies on the action of metrazole on autonomic responses, and Barry (14) has observed considerable species variations in the sensitivity of autonomic nerves after the administration of caffeine. Other pharmacological studies along these lines have appeared (8, 124, 135, 166, 287, 306).

A "law of denervation" has been stated by Cannon (44) based on the sensitization to chemical agents of smooth muscle, glands, skeletal muscle, and nerve cells, ganglionic and central, when partially or completely excluded from their normal nerve connections. The sensitization is particularly marked when the natural stimulating agent is used, but it is also obvious when other chemical substances are tried (190). Cannon (44) thus gives a new interpretation to Hughlings Jackson's concept of a hierarchy of functions within the central nervous system, and to the exaggerated responses which occur when lower levels are "released" from dominance from above. The partially denervated nictitating membrane in cats responds to large doses of epinephrine as does the normal control, whereas it reacts to small doses as does the totally denervated control, results which, according to Klopp (180), indicate that only some cells of the nictitating membrane have become more sensitive, the others retaining their normal sensitivity.

Bender (16) has added further data bearing on the differences in autonomic response in the monkey and the cat. Both adrenergic and cholinergic humoral effects take place in response to insulin hypoglycemia, the adrenergic phenomena predominating in the cat (18). Using the completely denervated iris of the cat as an indicator of circulating autonomic substances, Bender (17) found evidence of autonomic cholinergic substances circulating in the red blood corpuscles, but not in the serum, of the human and certain animal species. The denervated iris of the monkey did not show the same reactions. The slighter autonomic effects in the monkey in these various experiments may be explicable on the basis of a relative insensitivity of the indicator or of a fundamental difference in autonomic response in different species.

CENTRAL AUTONOMIC PATHWAYS

The medullary respiratory center, defined physiologically by stimulation, has been subdivided by Pitts, Magoun & Ranson (255, 256) into an inspiratory center localized particularly in the inferior reticular nucleus, and an excitatory center in the dorsal reticular formation. The spinal projections of these centers pass into the cord, chiefly ipsilaterally, through the anterior and antero-lateral columns where the vasomotor (91) and sudomotor (208) pathways seem to lie. Other autonomic responses, particularly pressor and depressor, to electrical stimulation of the brain stem have been studied (31, 238, 314). Wang & Ranson (314) have been able to plot out the descending autonomic pathways, which appear to run mainly in the lateral rather than the medial reticular formation of the lower brain stem. In the medulla the pressor and depressor responses become of much greater magnitude, suggesting an additional factor at this level. The central vagal inhibition of inspiration has been analyzed by Boyd & Maaske (26). The occasional acceleration of breathing by cooling the vagus has been attributed by Partridge (251) to stimulation of afferent cardiac fibers. Although reciprocal interaction of the cardiac accelerator and inhibitory centers appears to be the most common means of accomplishing the rapid cardiac adjustments which are demanded by changing bodily requirements, cerebral anemia, according to Hodes (156), causes this reciprocity to be lost, and in sympathetomized cats, which have fainted under stress, asphyxial excitation

of the cardiac vagal centers may slow the heart during such a period of collapse.

So many excellent reviews of the anatomy and physiology of the hypothalamus have recently appeared (23, 33, 53, 119, 164, 258) that it would be unnecessary repetition to review this work again. Only papers appearing since the publication of "The Hypothalamus" (*Research Publications of the Association for Research in Nervous and Mental Diseases*, 20, 1940) have been particularly considered. Special mention however must be made of the observation of Hare & Geohagan (129), and of Bronk and his associates (29), that stimulation of a restricted area within the central nervous system can give quite opposite effects (excitatory and inhibitory) when different frequencies of stimulation are used. To this may be attributed much of the contradiction in experimental evidence in the past. The necessity of defining the exact conditions of excitation is more apparent than ever.

The response of the gastrointestinal tract to hypothalamic stimulation has recently been reviewed by Sheehan (281). An excitatory effect on gastrointestinal motility following stimulation anteriorly in the hypothalamus has now been confirmed by Ranson and his colleagues (313), but the response does not appear to be a simple vagal effect. Hess (147) has again confirmed that stimulation of the "anterior" part of the hypothalamus leads to pupillary constriction, whereas stimulation posteriorly and more laterally gives rise to dilatation. From available evidence it does not however appear justifiable to assume that a parasympathetic center is being stimulated in such experiments.

The relation of the hypothalamus to the hypophysis, the effects of hypophyseal stalk section, and its role in water metabolism have been discussed in the section on innervation of the pituitary and thyroid (p. 419). Lesions in this area in guinea pigs have been followed by sterility and other disturbances in reproductive functions in many instances (73), though Dempsey (70) had reported negative findings in this regard. The recent study of the blood supply of the supraoptic and paraventricular nuclei by Finley (90) has revealed no evidence of a portal circulation between the hypophysis and hypothalamus, which had previously been postulated. Local heating by diathermy of the anterior hypothalamus in dogs caused inhibition of shivering and vasodilatation (138),

which furnishes additional evidence for the theory that the center for heat loss is located in this region of the hypothalamus. Curiously, panting was not observed in these experiments.

Local heating of the posterior hypothalamus generally resulted in sleep, with little if any changes in the thermoregulatory mechanism (138). Harrison's experiments (132) in this regard resulted in somnolence when the lateral hypothalamic area was destroyed electrically but not when it was stimulated. The hypothalamus in Rasmussen's experiments however did not reveal any injury at post mortem examination. Harrison's results (132) lend no support to the theory that sleep is a phenomenon of active inhibition, but rather corroborate Ranson's view of a "waking" center, in the hypothalamus, which "when functionally inactive permits the decreased nervous activity characteristic of sleep." Somnolence can of course be produced by lesions elsewhere, in the subthalamic region, for example, with the hypothalamus intact (234).

Although autonomic functions, it is now generally agreed, are under control of the cerebral cortex, there is still some controversy over their representation within the cortex. In a patient with complete hemidecortication Williams & Scott (321) found peripheral vasomotor responses and galvanic skin reflexes equal on both sides of the body. Similar reports from Carmichael's laboratory (229), have cast some doubt on a localized cortical representation of the autonomic nervous system. The weight of evidence, however, both from experiments in monkeys (103, 104, 254) and from clinical observations in man (51, 177a) points to a localization in the precentral cortex, more particularly in areas 4 and 6 (Brodmann). Bailey & Sweet (10) have obtained inhibition of respiration, rise of blood pressure, and decrease in the tonus of the gastric musculature on stimulation of the orbital surface of the frontal lobe in both cats and monkeys.

The concept of an independent autonomic nervous system, though long since disproved, lingers often unconsciously in our present day thoughts. The choice of the word autonomic is no doubt partly responsible, for it suggests, as Langley readily admitted, "a much greater degree of independence of the central nervous system than in fact exists." Almost from this time (1898), one might say, there began accumulating in every field of activity evidence of a constant interrelationship between the visceral and

somatic spheres. In the peripheral nervous system we have adequate proof of viscerosomatic and somatovisceral reflexes of various kinds. Sahs & Fulton (271) have recently observed corresponding effects on somatic and autonomic reflexes when the spinal cord was transected in monkeys. In the central nervous system it is not always possible to separate the autonomic from the cerebrospinal pathways; even in the hypothalamus, Hinsey (152) has shown that stimulation gives rise, in addition to visceral responses, to somatic movements involving the head, trunk, and extremities, and this after degeneration of all corticobulbar and corticospinal fibers. From the cerebellum, long the sole domain of somatic function, Moruzzi (243) has obtained evidence of control of autonomic responses, a central inhibition of the bulbopontine centers of respiration and circulation by the paleocerebellar cortex, while the unity of visceral and somatic patterns of response in cerebral activity is now no longer disputed. One is left therefore with a concept of a single nervous system, physiologically speaking, where visceral and somatic activities are closely integrated, and where each is probably under a certain control by the other.

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DEPARTMENT OF ANATOMY
NEW YORK UNIVERSITY COLLEGE OF MEDICINE
NEW YORK

THE SPECIAL SENSES

BY ERNST BÁRÁNY, RAGNAR GRANIT, AND
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PART I. HEARING¹

BY ERNST BÁRÁNY

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Phasic or nonphasic stimulation of the organ of Corti; duplicity theories; the function of the external and internal hair cells.—In a review in 1935 Ranke (47) stressed the need of a duplicity theory of hearing. There are well-investigated phenomena, some of which, e.g., directional hearing, demand that the vibrations of the basilar membrane or the cochlear microphonics constitute the immediate stimulus, while others, e.g., high acuity at high frequencies, indicate that the vibrations in order to be effective must be rectified and integrated into a unidirectional stimulus, a whirl (5), a region of static pressure (46), or a chemical mediator (19).

The physical theories of cochlear function are divided on the same basis. Some are only concerned with the vibrations of membrane and fluid (21, 28, 50), others involve vibrations plus a unidirectional event in the fluid (5, 46). It seems probable that the latter are nearer to the truth. Ranke's (46) mathematical treatment is the most thorough. His monograph contains a wealth of stimulating ideas which are impossible to quote here. The skeleton of his theory is as follows. The movements of the stapes give rise to a travelling wave on the basilar membrane. At the basis, its wave length is large in comparison with the diameters of the scalae. Its velocity and wave length decrease towards the apex, however, because of the increasing width and decreasing tension of the membrane. When the wave length is no longer large in proportion to the scalae, the pattern of propagation is radically changed, most of the wave is reflected at the point of transition, while the rest travels

¹ This review was finished on September 2, 1940. At this date many foreign periodicals, published in May or even earlier, had not arrived in Upsala and Stockholm. Thus, probably numerous important papers have not been reviewed. The writer fears that this has seriously reduced the value of the article as an annual review. As a compensation he has, with the permission of the editors, gone back and discussed a few problems and papers which are not treated in the excellent review of Stevens & Davis (57).

towards the apex with decreasing velocity and wave length and increasing decrement. The site of the transitional region obviously depends upon the frequency. This is the mechanism of frequency analysis. It is not quite clear what actually happens in the region of transition, but Ranke makes it probable that v. Békésy's eddies would occur there and that a static pressure is exerted from both sides against the membrane at this point. He believes this static pressure to be the direct stimulus of the spiral organ (of Corti). As the membrane vibrates with large amplitudes between the windows and the transitional region, there is no difficulty in imagining an independent and simultaneous rhythmic stimulation of the proximal part of the spiral organ (41). Thus, Ranke's theory could furnish the physical basis of a duplicity theory.

An anatomical basis has been repeatedly postulated by Lurie—most recently in (40). The internal hair cells rest on bone, the external on the membrane. The internal hair cells obviously do not vibrate as readily as the external hair cells, accordingly violent stimulation damages only the external hair cells. The internal hair cells are innervated radially never more than two cells to one fiber. The external hair cells are joined together by spirally arranged fibers innervating many external hair cells at different levels of the cochlea. In animals without external hair cells the threshold of cochlear microphonics is raised 30 to 40 db. At low intensities, only five-hundred pitches are distinguishable, against fifteen hundred at high intensity. Lurie concludes that the hearing of weak sounds is mediated by the external hair cells, precise pitch analysis by the internal. Nothing is assumed about a difference in the mode of stimulation, vibration being the only one of possible alternative stimuli considered.

A duplicity theory assuming phasic stimulation of the external and nonphasic stimulation of the internal hair cells has been put forward by de Maré (41, 42). He has discovered what seems to be an adaptation of the internal hair cells to constant pressure. His main experiment is arranged as follows: Loudness balance is obtained between two consecutive pure tones, each lasting 0.4 sec., presented to alternate ears. The pair recurs indefinitely after a pause of 2.7 sec. If the pause in one ear is filled up with a "fatiguing" tone of adjacent frequency, the loudness of the short tone in that ear immediately drops. With all tones at a sensation level of 88 db., the drop for a frequency difference of 30 at 500 cycles is 30 to 40 db. The effect decreases with increasing frequency intervals or

time intervals between fatiguing and test tone, and when the length of the fatiguing tone or its intensity is reduced. The effect is also noticeable, but to a lesser degree, as a threshold elevation.

Two findings deserve special mention. First, the rapidity with which the effect builds up and vanishes. A pause of 0.7 sec. between fatiguing and test tone reduces the effect from 30 to 6 db. The building up seems to be equally rapid. This time course does not resemble true fatigue but suggests adaptation of an end organ to a constant stimulus. Second, the effect is symmetrically distributed on the basilar membrane around the fatiguing frequency. There is no peak corresponding to the second harmonic even at 88 db. sensation level and 256 cycles. The symmetry is inexplicable if the effect has to do with the external hair cells with their unsymmetrical innervation or with the vibrations of the membrane, which are clearly unsymmetrical as revealed in the masking audiogram of a pure tone. On the other hand, the effect would be symmetrical if it had to do with the symmetrically innervated internal hair cells and the symmetrical distribution of constant pressure in v. Békésy's or Ranke's theories. This constant pressure could stimulate the internal hair cells in spite of their inability to vibrate. De Maré concludes that the external hair cells are stimulated by a phasic stimulus, probably the vibrations of the basilar membrane or of the fluid. The internal hair cells are stimulated by a nonphasic stimulus, probably the static pressure of Ranke and v. Békésy. The internal adapt rapidly. The external, being stimulated by a phasic stimulus, do not adapt. The internal are concerned with the hearing of pure tones. The subjective harmonics are heard with the externals.

In favor of this theory findings in pathological cases and several well-known physiological phenomena are quoted.

Experiments similar to those of de Maré (41) are reported by Nichols & Firestone (44). They investigated in three subjects the pattern of shift of threshold with frequency on exposure to a fatiguing tone of 202 cycles. One showed peaks of fatigue at 202 and 404 cycles, the others constant fatigue at 202 cycles and higher. Too few details are given in the short abstract to permit comparison with the findings of de Maré (41).

If the ear is exposed to a loud tone for 15 to 120 sec. the difference limen for intensity is least at the "adapted intensity." This is ascribed to central causes (49). The effect spreads about one to two octaves to each side of the loud tone. This large spread speaks

against a relation between this effect and that observed by de Maré.

The residue.—A phenomenon which seems to be explicable only by a duplicity theory has been described by Schouten (52). Using a photoelectric siren he produced a periodic impulse of 200 cycles very rich in harmonics. With an adjustable second 200-cycle tone he subjectively cancelled the fundamental, treating it in the same way as a subjective harmonic. He was surprised to find the pitch of the remaining mass of harmonics still at 200 cycles. Removing the compensating 200-cycle tone he was able to hear simultaneously a weak pure tone of 200 cycles and the sharp loud mass of harmonics also at 200 cycles. If the intensity was raised, the pitch of the sharp note remained practically unchanged, the fundamental showing the usual drop of about half a tone. Several other experiments are described which lead to the conclusion that the mass of higher harmonics, which Schouten calls the residue and which cannot be further analysed by the ear, is perceived with a pitch corresponding to the periodicity of its total wave form. Obviously, this is not in accordance with Ohm's law. A connection with the duplicity theory of de Maré (41) is the fact that here too we have a simultaneous but separate perception of a pure tone and something which must depend upon the vibrations in the proximity of the windows.

The above experiments are made with harmonics which are exact multiples of the fundamental. In the experiments of Jeffress (35) with organ pipes, the pitch of a complex tone without fundamental was more often judged to be equal to the second harmonic than to the fundamental. As a possible explanation for the disagreement with the well-known experiments of Fletcher, the author among other things refers to the fact that frequently the harmonics of musical instruments are not exact multiples of the fundamental. This explanation seems to fit well into the theory of residue, because in such a case the periodicity of fundamental frequency in the residue might be markedly reduced.

Pitch and loudness.—During the last five to ten years Fletcher and his associates in the Bell Laboratories have developed methods for measurement and calculation which have carried the psychophysiology of pitch, loudness, and masking a very large step forward. A synthesis of this work is embodied in the diagrams called "auditory patterns" (24) which show how much every segment along the basilar membrane contributes to the loudness of a sound

under varying conditions such as silence, masking noises, and deafness of conductive or perceptive type. In general an auditory pattern is calculated from the masking audiogram with the aid of two relations: (a) that between frequency and "nerve position" and (b) that between masking in a certain frequency region and the loudness contributed by the nerve endings corresponding to this region. Only the first relation will be discussed below; the second has been reviewed recently (57).

The nerve position of a point on the basilar membrane is the percentage of nerve endings between the point and the helicotrema. The relation between frequency and nerve position is obtained by integration of density data, giving the density of frequencies on nerve position as a function of frequency. The real problem is to obtain this density function. Three methods are used.

(a) It is assumed that a minimum audible pitch change corresponds to a displacement on the basilar membrane always comprising a constant number of nerve fibers. Dividing the pitch limens in cycles by this number, the frequency density is found. This is the classical method of Wegal & Lane.

(b) The efficiency of thermal noise in masking pure tones is used as a measure of frequency density (22, 23). This efficiency, expressed in the ratio of the intensity of the tone to the intensity per cycle of the noise necessary for masking that tone is larger at high frequencies. This indicates that at high frequencies even rather distant frequency components in the noise are located so near to the position of the pure tone on the basilar membrane that they contribute to the masking. If one assumes that this "critical region" around the position of the pure tone comprises a constant number of nerve fibers, the intensity ratio is a direct measure of the density of frequencies on nerve fibers.² The agreement with the pitch limen data is excellent.

(c) The width of a band of thermal noise around the masked tone is increased until no further increase of masking occurs. Here, obviously, we have a direct measure of the number of cycles per "critical region" and thus per nerve fiber. Again, the data are in good agreement with those of the other methods. The critical band width is twenty pitch-difference limens and corresponds to about 0.5 mm. on the basilar membrane (24).

The integration of these density data gives a very good agree-

² The concept of "critical region" is implicit in Fletcher's theory as Holtsmark (31) has pointed out. It is introduced here to facilitate the explanation.

ment with the results of animal experiments. There are, however, objections to the pitch-difference limens of Shower & Biddulph (54) used in method (a). These values, at least those up to 1,000 cycles, would have been obtained even with a perfect ear because of the principle of uncertainty (37). Consequently, the actual difference limens must be smaller than those experimentally found and the density below 1000 cycles calculated must be too high. This gives the low frequencies too small a part of the basilar membrane (29). In view of this criticism, the excellent agreement between the results of the three methods is puzzling. It would be explained if the independence between them were only apparent. Treating a model of the basilar membrane consisting of a series of independent, only slightly damped resonators, Holtsmark (30) shows that in this special case the first two methods are in certain respects mathematically equivalent, since from an experiment based on method (b) he derives the equation behind method (a). It remains to be seen whether this equivalence is obtained even with a more realistic assumption about the mechanism of frequency analysis in the cochlea. The close relation between methods (b) and (c) is obvious from the presentation given above.

In this discussion, only one problem treated in Fletcher's papers (22, 23, 24) has been touched. From his theory, there result a great many interesting and physiologically significant quantitative relations between excitation, stimulation, loudness, masking, etc., which must be studied in the original publications. To the reviewer, a specially puzzling fact is the successful use of the concept "nerve position" without difficulties arising from the spiral innervation of the external hair cells. On the whole, a connection between the work in the Bell Laboratories and the duplicity theories is badly needed.

The perception of a pure tone as such in spite of the widely spread response it evokes on the basilar membrane makes it necessary to assume inhibition mechanisms acting upon the neurons on each side of the one maximally stimulated. In pathological cases, this mechanism could be damaged. One would then expect an increase in loudness and the perception of pure tones as noise. This is the explanation given by Lorente de Nó of an interesting discovery by Fowler (25). In certain nerve-deaf ears he has found that at high intensities the sick ear actually has the louder perception and that the tone is perceived as a noise.

These observations are intimately related to those of patho-

logical pitch perception ("diplacusis"). When pure tones are perceived as noise the perceived pitch often is different from that of the exciting tone (cf. 3). In such cases, diplacusis obviously has no simple mechanical explanation. On the other hand, it is very probable that pathological states, such as edema, of the vibratory structures could change the mechanical constants and give rise to altered distribution of frequencies on the basilar membrane. This is the view favoured by Shambaugh (53) who presents a large series of carefully studied cases.

Transients.—If the cochlea consisted of a series of tuned resonators the response to a transient would be exactly predictable from its Fourier spectrum. There are, however, very good reasons to believe that the cochlea functions in quite a different way and it is not at all certain *a priori* that this will include analysis by the method of Fourier (cf. 52). Deviations from a Fourier behaviour which would be of great theoretical significance would probably be most easily detected in a study of transients, which therefore ought to attract more attention than hitherto.

Although it does not touch the above question the work of Türk (58) is of physiological interest because it contains a very good method for producing transients, a study of the audibility of different building-up and decay characteristics of pure tones and new determinations of the minimum duration for pitch perception. Using tones of relatively gradual onset the duration thresholds are much smaller at high and low frequencies than those of abrupt onset.

Subjective tones and distortion of the cochlear microphonics.—Wever, Bray & Lawrence (59) use a new method for eliminating the middle ear. The stapes is directly driven by a vibrator. The harmonics in the cochlear microphonics do not differ significantly from those in the normal ear and thus probably are generated in the cochlea. In these measurements, action potentials could play a role. If they are eliminated by denervation of the ear (48), the harmonic content is without doubt a measure of distortion. There is a difference as compared with the normal ear which is not very great except at low frequencies.

The relation between subjective harmonics and those in the cochlear response is uncertain. For instance, the dependence upon amplitude is different. Schouten (51), using an improved method, has found that the relative intensity of the subjective second harmonic rises rapidly with the amplitude of the fundamental. This is

not the case for the cochlear response in the denervated ear (48), where the relative intensity of the second harmonic is constant over a large intensity interval.

Schouten's new method consists of an adjustment of the exploring tone to minimum roughness and to minimum beats with a second exploring tone. It gives values for the second harmonic very much below the "best beats" method. Lewis (39) defends the sole use of an exploring tone in cases where the subject actually hears the harmonic to be cancelled.

Directional hearing.—A monograph covering a large part of this field has been written by Wilska (60). Only a few of his results can be mentioned. Using sounds of various quality he determined the acuity of directional hearing, which by analogy to vision he defines as the least angle with which the ear is able to distinguish the direction of two successively sounded sources. His figures show the importance of distinct and frequent "time marks" for direction hearing and are in excellent agreement with the time difference theory. Noteworthy is the very high acuity when a mixture of two tones is localized and the finding that 52 of 57 subjects were unable to perceive "*Drehtöne*" at c_1 if intensity fluctuations were eliminated.

Hughes (33), working with pure tones, found the rise in least audible phase difference caused by the refractory period of the nerve to occur at about 1,000 cycles. This is in accord with expectation.

Perception of distance.—A thorough analysis has been made by v. Békésy. Distance of clicks is monaurally perceptible not only with the ear but also with a microphone or the orifice of a sound conduct as reference point. Thus, the relation of the sound field to the head can not be the determining factor. The clue to distance must lie in the sound field *per se*. In the sound field from a point source of the Oth order, the ratio of pressure to velocity grows with distance. In clicks, pressure contains louder high-frequency components and fainter low-frequency components than does velocity and v. Békésy shows that it is the ratio of high to low frequencies in the noise which determines the monaural judgment of distance. This is often very definite but at the same time quite wrong, if the noise is not well known beforehand (6). If the noise is very well known, as is the case for speech, the distance is so accurately judged and discounted that it is more easy to adjust two unseen loudspeakers at different distance to equal output than to adjust them to equal loudness at the point of observation (43).

The absolute intensity threshold.—Although the problem has not been explicitly stated, several recent papers have a bearing on the question of whether the threshold is determined by (a) an all-or-nothing mechanism in the periphery, (b) a threshold of flow of impulses to the centers, or (c) the masking level of subconscious noise. Of course, all these alternatives could be true in different frequency regions. The staircase audiogram at very low frequencies (8) indicates that alternative (a) is at work, as does the finding that strong suggestion of increased acuity in hypnosis did not lower the threshold at 925 cycles (56). On the other hand Hughes (32, 34) finds that subliminal stimuli in one or both ears are added without energy loss regardless of frequency relations. This speaks strongly for alternative (b), which also receives support from the finding that patients with different hearing loss in both ears have a lower binaural than best monaural threshold (27). Against a peripheral threshold speaks also the probable existence of intersensory summation: the threshold at 1,000 cycles is lowered by a preceding flash of light (12).

That thermal noise in the air could be the limiting factor at the region of highest acuity was suggested by Sivian & White (55). Fowler in an excellent paper on tinnitus states that it is possible to hear this noise in a sound proof room (26). There is, however, inevitable noise other than the thermal. Regarding the ear as a sensitive microphone, one is surprised at the crudity of its suspension. In the immediate vicinity and without proper insulation, fluid and gas are rushing through tubes, and muscles in continuous low frequency vibration are attached at the casing. This "physiological noise" is easily perceived with plugged canals (55) but might very well be the limiting factor also in normal hearing. It is perceived by bone conduction which gains new physiological significance from this fact (4).

Audiograms for dog and cat are given by Dworkin *et al.* (20). They also describe methods for conditioning and testing. Both species had remarkably good hearing for very high frequencies.

Bone conduction.—The physiology of bone conduction has recently made advances through new methods of measurement, eliminating the variable influence of the soft tissues covering the skull (4.7) and permitting the measurement of phase as well as amplitude of the bone-conduction tone (4). V. Békésy (7) has developed piezoelectric and electrostatic pressure-sensitive elements which do not respond to their own movements and has made

the first bone-conduction audiograms in terms of vibrational force as well as a study of the sound transmitting properties of the skin.

Vibrators transmitting a force of constant phase and amplitude to the skull independent of the skin have been built and used in a compensation method for mapping the distribution of phase and amplitude of bone-conduction on the head and studying its various components (4). The air sound from vibrator and head was measured separately and vectorially subtracted from the bone-conduction tone. At opposite sides of the head the phases of the remaining true bone conduction in normal ears at 435 cycles differ about 180° , the amplitudes are approximately equal. This shows that the movements of the head and not the pressure wave through it are the main source of bone conduction at this frequency. The distribution of amplitudes suggests that the dominating component is generated by rotatory inertia oscillations of the ossicles around their axis. The amplitude of these movements is proportional to the degree of unbalance of the chain around its axis. In accordance with the view that bone conduction is harmful the curious and clumsy shape of the ossicles is explained as an attempt to balance the ossicular chain. The new methods were also applied to a study of bone conduction under abnormal conditions in the middle ear. With the aid of middle ear reflexes it has been shown that some bone conduction remains even after extirpation of the stapes (36).

Auditory tracts.—Coppée (15) has shown that in the rabbit there is only a crossed path, in the cat a crossed and an uncrossed. Using various methods (14) he demonstrates spatial differentiation of frequencies in the posterior quadrigeminal bodies. An interesting result is that a warbled tone gives very large action potentials in the centers. This is explained as a succession of on and off effects in neuron chains from the cochlea and is used (16) for an objective determination of pitch difference limens. In the cat this is 2 per cent, in the rabbit 4 per cent.

A projection of the cochlea seems to be certain even in the medial geniculate body of the cat, where it has been studied by Coakley & Culler (13) with the aid of action potentials. Even the frequency of the stimulus was reproduced at this level. Previously, Culler *et al.* had demonstrated projection with the aid of localized lesions (1).

Bremer (9), studying the cortical acoustic area of the cat, found responses to be bilateral but stronger at the contralateral

side. The response shows rapid fatigue but recovers on frequency change. This suggests new neuron chains coming into play. It has not been possible to find any other indication of spatial differentiation between frequencies in the cortex. The acoustic area delineated by electric methods is in good agreement with cytoarchitectonic data (10).

Indirect evidence for a central spatial differentiation of frequencies in man is given by the demonstration of unmaskable "central" tinnitus besides the maskable "peripheral" (26).

The latent period of the crossed stapedius reflex in man is 10.5 msec. (45).

Differences between the acuity of left and right ear do not seem to be correlated with left- or right-handedness (11).

A number of papers are concerned with the human electroencephalogram in relation to auditory stimuli (2, 17, 18, 38). It seems still to be too early to attempt a correlation between these findings and the physiology of hearing.

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THE SPECIAL SENSES

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PART II. VISUAL RECEPTORS

BY RAGNAR GRANIT

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In two consecutive reviews in this journal Olmsted (61, 62) has reviewed the literature on the eye up to September 1939, therein discussing also such subjects as eye movements, pupil, lens, and ocular fluids. This being so, the present author feels that he is doing more justice to the intentions of the editors and to the present situation¹ as well as to his own special background if he concentrates on a more limited field.

Some recent summaries.—Many of those engaged in visual research have recently summarized our knowledge in different branches of this field. Very comprehensive, especially with regard to contributions from zoological quarters, is a book by von Stüdnitz (78) who also has published an article on the duplicity theory (79). A general review of some recent experimental work has been written by Wright (95), whereas summaries by Lythgoe (52) and Bartley (2) have the character of analytical discussions of the mechanism of dark adaptation (Lythgoe) and neurological aspects of visual reactions (Bartley). In the introduction to his work on the regeneration of visual purple Zewi (97) supplements the author's (28) review, "Processes of adaptation in the vertebrate retina in the light of recent photochemical and electrophysiological research," with regard to the photochemistry of visual purple. Wald (89) has given an account of his work on the chemical aspects of visual purple decomposition, and Stiles (75) summarizes the results of his own and Crawford's research regarding the curious "directional sensitivity of the retina" that they have discovered.

Photosensitivity of visual purple.—The newest contribution to

¹ Latest periodicals from the U. S. A., of those which have been accessible at all, are those published in July 1940; British periodicals since April 1940 have practically ceased to arrive.

this question (70) is one which provides the author with an opportunity of paying a tribute to the memory of a fellow worker, Dr. R. J. Lythgoe of University College, London, a leading expert among the devotees of the eye, who died in the spring of 1940. A thorough knowledge of the subject of vision, scrupulous criticism, accuracy, and experimental initiative guided his research, part of which was carried out in collaboration with the photochemist, Dr. Goodeve. Among Lythgoe's recent contributions should be mentioned the first accurate absorption curves at different pH levels for the yellow, relatively stable indicator substance (Kuehne's "visual yellow," Wald's "retinene" or a substance closely related to it) into which visual purple in solution decomposes upon illumination (51); the first accurate measurements of the extinction coefficient for visual purple (51), and of the quantum efficiency (about 1.0) of this photochemical reaction (23), which has been analysed in several papers; and the discovery of the interesting "transient orange" photoproduct (51, 53) which is the initial, very labile stage of visual purple decomposition. In the latest experimental work (70) the photosensitivity is measured directly in terms of the product of the extinction coefficient and the quantum efficiency for a number of wave lengths. It was found that the photosensitivity actually runs parallel with Lythgoe's earlier standard curve for the extinction coefficient alone, a fact which definitely proves that the large values for visual purple absorption in the blue end of the spectrum do not depend on admixture of impurities. In the meantime Ludvig & McCarthy's (50) measurements of the light lost owing to selective absorption in the extra-retinal part of the eye have appeared, showing light from the blue end to suffer severe losses on its passage to the retina. Their work finally explains (49) why the sensory (44) and the electrophysiological data (26, 27), obtained with an intact eye, have fallen inside the absorption curve for visual purple in the blue end of the spectrum. Such comparisons nowadays presuppose correction of the data on the basis of equal quantum efficiency rather than on equal energy, as was first pointed out by Dartnall & Goodeve (22).

Actually, the frog's eye may at higher intensities give larger electrical responses to blue and violet than would be expected on the basis of its average visibility curve for low intensities, a fact noted by several observers. [See author's recent summary (28), p.

48]. We shall return below to the interesting question of "blue reception."

Two phases in visual purple regeneration.—Zewi (97) has published an extensive analysis of the regeneration of visual purple in live frogs, based on over one thousand five hundred eyes. Curves illustrating dark adaptation in terms of visual purple concentration plotted against time showed the effect of temperature on the regenerative processes to be large. Very interesting seems the observation that the slow course of regeneration in intact animals at low temperatures was duplicated by the equally slow course of regeneration in excised eyes. In the latter case regeneration was independent of temperature. In other words, that part of the regenerative process which is sensitive to temperature is lacking in excised eyes. It presupposes live frogs. Short times of preadaptation with strong light led to an initial period of delay in the regeneration of visual purple. None of Zewi's regeneration curves could be fitted into one of the well-known types of chemical reaction. Von Studnitz (80) has emphasized the significance of the oil globules for regeneration.

Illumination enhances regeneration of visual purple.—Another result of Zewi's work was the observation that illumination of the frogs speeds up regeneration of visual purple. A step further along this road is reported in a paper by Chase & Smith (12), continuing work begun by Chase (11) dealing with the old subject of regeneration in solution. Under these conditions there is relatively little visual purple formed but the process seems to take place without changes in the absorption spectrum of the substance. In view of the results mentioned above, one must presume that only one phase of the regenerative process, the one found in excised eyes, can be concerned. In accordance with this supposition stands the fact that regeneration in solution is independent of temperature. Now this phase of regeneration was found to be enhanced by previous illumination of the solution with blue light. The maximum of the effect was located somewhere between 0.490 and 0.440 μ . The authors have not been aware of the fact that a similar favorable effect of previous illumination with blue light upon the regeneration of the retinal electrical response of the frog has also been demonstrated (34). Visual purple regeneration may be stimulated by decomposition products of its own breakdown or by some other blue-sensitive substance which may or may not have other

tasks to carry out in the sensory mechanism. To quote Lythgoe (52): "One cannot help wondering whether the photosensitive substances responsible for day vision are formed in this way: the bright light may be the essential agent in the preparation of the visual purple for the reception of high illuminations." The subject is further considered by Granit *et al.* (34). Actually the visual purple of the living animal is never completely bleached away (32, 52, 97).

Oxygen and visual purple regeneration.—Recent results with sensory methods have shown that dark adaptation was disturbed during oxygen deprivation in a low-pressure chamber (55) or during inhalation of gaseous mixtures with low oxygen content (54). The results were held (55) to be due to an effect on the central nervous system rather than on the photochemical substrate. Now neurons are known to be sensitive to lack of oxygen; Hartline (37) has shown that the eye is no exception. Similar results in experiments on visual acuity (56) in a low-pressure chamber also lend themselves to such an interpretation. In these experiments red light was used and hence stimulation of visual purple was insignificant. Nevertheless it should be emphasized that visual purple regeneration is exceedingly sensitive to oxygen lack (97). According to Lythgoe (52), "It seems possible that we have two methods for the regeneration of visual purple. Regeneration from its breakdown products almost certainly involves the addition of energy to the system, and this energy can be provided either by the absorption of light or by a chemical process needing oxygen."

The decomposition of porphyropsin.—In the rods of certain fresh-water fishes, the visual purple of amphibians and mammals, known as rhodopsin, is replaced by a purple pigment with a maximum in the region of 0.535 to 0.540μ (difference spectrum). For this substance the name "porphyropsin" has been suggested (Wald). According to Wald, who has summarized his work on this subject (89), decomposition of rhodopsin in the retina leads to formation of vitamin A. One of the intermediate decomposition products is a yellow carotenoid substance, Wald's "retinene," closely related to Lythgoe's "indicator yellow." Now it seems that the decomposition of porphyropsin also leads to a similar intermediate product with vitamin A₂ as the end stage (87). Retinene and vitamin A seem to be replaced by these two new carotenoids, retinene₂ and vitamin A₂, with slightly different ab-

sorption spectra. Vitamin A₂ is a recent discovery by the biochemists. A study of the distribution of these two vitamins in fishes (88) is perhaps less directly concerned with vision.

Visual purple; sensitivity to light; vitamin A.—In a recent review in Swedish (29), the author has criticized the view that sensitivity to light is merely the equivalent of concentration of visual purple. The experimental work behind this criticism consisted of parallel measurements of the electrical response to light and the concentration of visual purple (31, 32). Thus, for instance, after bleaching with 3400 m.c. leaving some 40 per cent visual purple, the electrical response of the cat's eye is reduced to next to nothing. On the other hand nearly maximal responses of the frog's eye may be obtained without measurable reduction in the amount of visual purple present. Lythgoe (52) has added other arguments, among them a comparison referring to the human eye: "There are no figures for the concentration of visual purple in the human retina at different periods of dark adaptation, but we will assume that the figures of Granit *et al.* (32) for the cat are applicable to man. Between the tenth and thirtieth minutes the concentration of visual purple increases from 57 to 95 per cent of its final value. According to the simple theory, the visual threshold should be divided by about one half (57/95) during this period, whereas it is, in fact, divided by about one thousand. In other words, the increase in concentration is not nearly enough to account for the great increase in sensitivity."

Similar objections are raised by him in connection with the question of the night blindness and vitamin-A deficiency. To Olmsted's list of references (61, 62) from this steadily expanding, but theoretically uninteresting field, should be added the newest ones (46, 59, 60, 66, 68, 84, 85, 86, 94). One can only wish that the demonstration by Johnson (46), confirming Tansley (81), that lack of vitamin A may lead to degenerative changes in the retina at an early stage should not be forgotten. Their results could explain why some authors (41, 66) find experimental hemeralopia to affect the cone system too and to be relatively resistant to vitamin-A therapy. That the vitamin somehow is involved in the mechanism of regeneration of visual purple (see above) need not be the only explanation of experimental hemeralopia worth considering.

Colour reception analyzed with microelectrodes.—This technique which recently has been used with some success in other fields by

Buchthal (10), and by Forbes and his collaborators (67, 83) as well as by Lorente de N6 (48) in the central nervous system, is particularly well suited for the analysis of spectral properties of single or a limited group of elements in the eye. Granit & Svaetichin (33) describe it in detail and give illustrations of the activity of single elements responding to different parts of the spectrum. Using the light-adapted frog's eye they compare the average spectral distribution curve for a great number of elements acting together with the distribution curves obtained from a single or a restricted number of elements. The average curve has its maximum between 0.555 and 0.560 μ and is made up of contributions from elements with much narrower sensitivity bands and different maxima. A definitely isolated element has its maximum between 0.600 and 0.580 μ . There is also a "blue" receptor with a maximum somewhere around 0.460 to 0.480 μ . The "green" receptor with a maximum between 0.500 to 0.530 μ may or may not be identical with visual purple. Precautions were, however, taken to exclude visual purple. Somewhat surprising was the fact that the element with a maximum of 0.580 to 0.600 μ after dark adaptation changed into one with the maximum of visual purple (around .500 μ) and the high sensitivity of the rods. Whether this means that a cone changes into a rod or that rods and cones go to the same final common path, which after dark adaptation is taken over by the rods, is difficult to say. The fact, however, is in itself of primary importance. The presence of a "blue" receptor confirms earlier results with the electroretinogram (28, 34, 35). A significant observation also was the striking rotation of activity among the different elements, which is a serious source of error in experiments of this kind.

Photochemical model of the retina.—In two papers Weigert has continued description of his "artificial retinae," gelatine films containing visual purple dissolved in digitonin (90, 91). In view of some of the results referred to above (11, 12, 33, 34, 35, 52), it is indeed interesting to find that illumination of such films with certain monochromatic lights leads to changes in the absorption spectrum. There are rather striking analogies with the behavior of the vertebrate retina, particularly those effects which are produced by mixing two monochromatic lights. The one article (91) describes in detail and with great clarity both the results and the technique of measuring the selective effects in the gelatine films

with the aid of photodichroism. To their paper the reader must be referred for instruction in this matter.

General observations on excitation and inhibition.—A most interesting report by Hartline (38) deals with the off-effect in single fibers of the optic nerve. A third of the fibers respond only to cessation of illumination or to a sudden reduction of intensity. This discharge is completely inhibited by reillumination, as indeed was to be expected. Hartline's concept of the "receptive field" (37) of a fiber of the optic nerve again proves its usefulness. The sensitivity within the receptive field as well as the boundaries of it can be mapped by moving a very small spot of light over it. The receptive field is most sensitive in its center. In accordance with this stands the fact that an off-response is obtained only when the spot of light is shifted to a less sensitive part of the receptive field, as well as after a sudden decrease of the illuminated area. Further, the greater the area reilluminated the more effective is the suppression of the off-discharge. "Thus there are inhibitory effects due to illumination which converge upon the ganglion cell from all portions of its receptive field and are summed in the total inhibition produced" (Hartline, 38).

In a paper by Granit & Helme (30) the electroretinogram and the impulses in the optic nerve, recorded during polarization of the retina, were described. The responses in both retina and nerve were found to be greatly enhanced when the inside of the eye was the cathode, and depressed when it was the anode. The components of the retinogram are discussed in this paper as well as in a valuable summary by Bartley (2) who also emphasizes the significance of the rotation of activity mentioned above.

Bernhard (4) has shown that during illumination of the eye, a slow potential change derived from the ganglion cells spreads electrotonically along the optic nerve. Some records from the frog's optic tectum illustrate potential changes in this region. Bishop and O'Leary (7) have also described potentials from the optic pathway. Simultaneous records by Bernhard of the human electroretinogram and the α -rhythm demonstrate that the blocking of the α -rhythm, following illumination, always takes place after a constant interval from the onset of the electroretinogram.

Crescitelli & Jahn (14, 45) have found notable diurnal changes in the electrical response of the compound eye. Very interesting is their observation that at sufficiently high temperatures the elec-

trical response of the grasshopper eye to short flashes of light ends with a series of rhythmic waves which are suppressed by increased duration of stimulation. Cooling the eye was found not to leave a negative component corresponding to that of the vertebrate eye.

In two papers by Wilska (92, 93) microrecording from the retina as in the colour work, described above, has been reported. His most noteworthy result is the proof that the inhibition of the off-effect found by Granit and by Hartline in cold-blooded eyes also exists in the cat's eye. In other respects his results are covered by Hartline's earlier work (37).

Measurements of the latent period of the *a*- and *b*-wave of the electroretinogram have been published by Piéron & Segal (64). From the fact that the latency of the *a*-wave is far less sensitive to cold than that of the *b*-wave, they conclude that the *a*-wave represents part of a more distally located "primary response" to light.

Therman (82) has determined the size of the electrical responses to red and blue light in the frog's eye in relation to state of adaptation. The response to blue increases in the dark, and diminishes during light adaptation while the response to red increases. The retinal pigment can be made to take up the "light adapted," expanded position after an injection of epinephrine. This, however, does not lead to the changes in the electroretinogram accompanying "real" light adaptation. The expanded pigment cannot therefore have exerted any protective action on the rods.

Some effects of repetitive electrical stimulation of the eye have been reported by Bouman (8) and Schwartz (72). In two papers Miles (57, 58) adduces evidence in favour of the view that the so-called polarity potentials of the eye are due to the current of rest, which changes during eye movements owing to the displacement of the bulbar poles relative to the electrodes applied on the skin outside the eye bulbs. Similar arguments are advanced by Lindsay & Hunter (47). See also Baudouin *et al.* (3).

Directional sensitivity of the retina.—By this term is meant the now well-known fact that a pencil of light reaching the cones at an angle is less effective in creating a perception of brightness than one entering them along their own vertical axes. Directional sensitivity is not found in rods, according to Stiles (75). The newest contribution by Stiles (77) shows that the effect is absent

in the parafovea until the eye has been light adapted and supposedly become dominated by cone vision. An interesting effect of wave length had previously (76) been noted in the form of colour changes as the angle of incidence of the beam was altered. The new measurements of the effect of the angle of incidence upon the difference threshold of a small foveal monochromatic patch, placed in a field of larger dimensions, both of which are variable with respect to wave length and intensity, show changes in directional sensitivity at different levels of brightness. These mimic the changes obtained in the parafovea when rod vision, owing to increased brightness, is replaced by cone vision. Stiles concludes that the effects on a pure cone population in an analogous manner are due to one type of cone being replaced by another. He gives a preliminary calculation of the spectral properties of the three types of cones assumed in order to explain the data. Perhaps one should remark that the experimental conditions imposed favour effects of interaction in the retina which may complicate evaluation of the results.

As to the explanation of the directional sensitivity Wright & Nelson (96) may be right in suggesting that it depends on differences in the refractive index between the receptors and the surrounding medium. Neither can Best's (5) view be neglected, that the absence of a directional effect in rod vision may be due to the capacity of the rods to summate and thus to collect all the light of the less perfect image of the tilted beam till it matches the brightness created by the central beam.

Some work with sensations.—By "indirect adaptation" Schouten & Ornstein (71) mean the fact that illumination of an adjacent or distant area depresses the brightness of a test field. The use of Wright's binocular method has greatly facilitated the study of such processes, as can be seen in this paper as well as in one by Pitt (65). There is α -adaptation which spreads almost instantaneously and soon depresses the sensitivity of the whole retina to an almost stationary level. During this period of stationary low brightness, something else in addition takes place in the retina, as is shown by the circumstance that the recovery curves after adaptation are slowed down by longer exposures. This delayed recovery is an expression of β -adaptation. After short exposures the α -process alone rises very quickly. It is held to be electrical in nature. For details concerning these and related facts the

reader is referred to the two very instructive papers mentioned.

From the point of view of retinal and central mechanisms measurements of visual acuity and brightness discrimination as functions of log brightness cannot deal with very different mechanisms. Yet in Hecht's (39, 40) older attempts to apply his well-known "photochemical system" it struck one that, in the equations for the rod and cone systems, the constants came out as 0.0906 and 0.794 respectively for "visual acuity," and as 100 and 0.25 for brightness discrimination. The absolute values are of no significance but it did not seem very reasonable that the ratio of rod to cone constants should be so absurdly different in the two cases. Hecht & Mintz (42) have now studied visual acuity under simplified conditions of visual resolution and give an interesting mathematical treatment of this problem in which the distribution of light over the retinal image is also considered. They state that the visual resolution and intensity discrimination now are described by the same equation. In view of the older results a comparison of the constants would have been worth having.

Crozier & Holway (15) present measurements of brightness discrimination and deduce an equation without reference to specific theory. In a number of papers (16 to 21) Crozier & Wolf report continuance of their work on the reactions to flicker in different animals, determining the point of fusion with the aid of behavioristic reactions. They claim that the curve for flicker-fusion against log intensity is a probability integral in log I , and state, in opposition to Hecht, that the physicochemical nature of the excitatory process cannot be deduced from measurements of this type. The authors have been trying systematically to elaborate a descriptive quantitative system based on the probability integral.

Graham and his collaborators (24, 25) have contributed to the old question of summation at the threshold relative to size of stimulus. An interesting new feature of the quantitative treatment of the data is their utilization of Hartline's concept of the "receptive field" (see above). Bartlett & Gagné have tested binocular summation at the threshold with negative results (1). Visual thresholds have also been studied by Chevallier & Roux (13).

The blind spot is monographically treated by Bröns (9) who also adduces experimental evidence to the effect that the filling-in of this physiological scotoma is due to special connections of the

nerve fibers from the parapapillary zone. See also Saubermann (69). A thesis by Olsson (63) deals with the colour changes of a test field as it moves towards the periphery. His results lead him to dispute the existence of the so-called yellow macular pigment.

Interesting from the point of view of retinal physiology is the demonstration by Smith (73, 74) that in cats the reactions to apparent and real movement persist after all somatic cortex has been removed. Animal behavior is also used by Birukow (6) and by Grether (36) for the analysis of the colour sense of frogs and chimpanzees respectively. For the same purpose Hecht & Pirenne (43) have used the iris muscle contraction to establish that the visibility curve of the owl is consistent with the idea that its scotopic photochemical system is visual purple.

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THE SPECIAL SENSES

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PART III. VIBRATORY SENSATIONS AND PAIN

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VIBRATORY SENSATIONS

An excellent historical survey of the nearly continuous controversy of opinion regarding the nature of the process underlying the perception of mechanical vibration has just been published by Geldard (13). Recent technical advances, particularly the development of the vacuum tube oscillator, by means of which well-controllable stimulating devices are made available, has no doubt encouraged research in this field, and led to a revival of the old debate as to whether vibratory sensations are mediated by the same end organs responsible for contact and pressure, by deeper lying receptors, by a combination of both, or whether there is a separate vibratory sense. Furthermore modern electrophysiology has offered means of a direct study of the activity of the nerve fibers responding to vibratory stimuli. Nevertheless, as stated by Geldard, the literature of the last decade has advanced more new facts than new ideas for the solution of the old debated problem.

In a series of new experiments, Geldard (14) provides further evidence for the view that "vibratory sensations fall into a perceptual pattern of feeling of which pressure is but another temporal expression." Using a method which allows an accurate observation of the amplitude of the vibrating needle, he measured on the forearm above the wrist the thresholds of two selected populations of skin spots, one of high pressure sensitivity, the other unresponsive to relatively strong mechanical pressure. The pressure-sensitive spots were found to have an average threshold of 13.6 micra, whereas that of the pressure-insensitive spots was over ten times as large. In a following paper (15) Geldard has reported the thresholds of the two spot populations at five different frequencies: 64, 128, 256, 512, and 1,024 cycles. The pressure-sensitive spots yielded low and reliable vibratory thresholds; spots insensitive to pressure gave high and variable values. The frequency

function for the sensitive spots was practically flat in contrast with all previous determinations of the frequency-amplitude relation. Thus, regardless of the frequency of stimulation, vibratory thresholds of local spots which have high sensitivity to pressure remain constant. This fact is taken as evidence for the view that the amplitude of movement, or size of deformation, not energy, is the stimulus correlate of intensity of vibratory sensation. This opinion is supported by some recent experiments by Békésy (5). A finger kept immersed in paraffine oil under a pressure of 70 gm. per sq. cm. was exposed to vibration of 200 cycles. Although the sensitivity in the finger top was two neper larger than in the other parts of the hand, the vibrations were felt only at and above the border of immersion. As in the case of Meissner's experiment this observation shows that the production of a pressure gradient is necessary for provoking vibratory sensations. From measurements of the frequency dependence and of the mechanical impedance of the skin Békésy arrives at the same conclusion, namely that deformation of the tissue is the adequate stimulus.

During the last few years the effects of local anesthesia on the vibratory sensitivity have been studied by several investigators. Cohen & Lindley (9) observed that ethyl chloride spray lowered the threshold, while cocainization by intradermal injection did not provoke any change. Cummings (10), using Rein's iontophoretic method, found a slight rise in the vibratory threshold but it was very small in comparison to the very definite decrease in tactile sensitivity in the same area. Weitz (35), however, found a marked rise when the skin had been anesthetized electroendosmotically. Studying the recovery of sensitivity after procainization he found that prick sensitivity returned first, but was not accompanied by any change in vibratory sensitivity. With the return of "contact" sensitivity a marked drop was noted in the vibratory thresholds, and they returned to normal with the recovery of "pressure." Newman, Doupe & Wilkins (27) also found that superficial cocainization by electroendosmosis raised the threshold for the vibratory stimulator when this was making light contact with the skin but that it was unchanged with heavy contact. They also report that the vibratory sensitivity to both light and heavy contact is unchanged after tractotomy when the pain-mediating spinothalamic tract is sectioned. Vibratory sensations to heavy contact were found abolished in a case with a typical Brown-Sequard syn-

dromé, where appreciation of passive movements was defective. As vibration, touch, and pressure are the first sensations to be affected by the application of pressure to the nerve but disappear comparatively late when novococaine was the anesthetizing agent they assume that the fibers subserving vibratory sensations are of the same size and similar to those mediating touch and passive movement.

By means of measurements of great precision of punctiform stimulation, Békésy (4, 5) found points which, though of constant sensitivity for pressure, differed more than sevenfold for vibratory sensitivity. By regulating the amplitude of vibration of the hair stimulus used, the sensation of pressure could often be completely masked by the sensation of vibration, while only 0.5 mm. away from this point of stimulation no sensation of vibration was felt at all but only the pressure sensation caused by the initial pressure of the hair stimulus. It was found that the points for pressure and vibration do not coincide in space, but that the point for vibration is a little more distant from the place of entrance of the hair into the skin than is the point for pressure, both spots lying exactly above the position of the hair in the skin. The conclusion was drawn, therefore, that the endings for the sensations of pressure are to be found directly under the sebaceous gland, and those for the sensation of vibration near the hair papilla corresponding to the position of the two sets of nerve endings which have been described by Retzius and others at the hair roots.

In this connection it is worth mentioning that Becker & Fröhle (3) present evidence that the tactile sensibility of the skin is not limited to that of the touch spots. Using Endres' method for production of punctiform anesthesia they succeeded in producing an isolated anesthesia (*a*) of a number of adjacent touch spots, and (*b*) of the skin between the spots without affecting the touch spots. The threshold to punctiform stimuli in the region of skin free of touch spots was considerably raised by both procedures. Accordingly they assume that the skin is supplied by tactile fibers which course out from the touch spots to the superficial layers of the surrounding skin in accordance with recent histological observations.

An important contribution to the solution of the question of the mediation of vibratory sensations from deep-situated tissues has been made by Echlin & Fessard (11). They found that a vibrating

tuning fork applied to the bones or tendons of animals elicited rhythmic discharges of impulses synchronized to the stimulation frequency. These discharges were shown to derive from stretch receptors in the muscle. The individual composite waves recorded from the nerve were found to be the result of summation of impulses from many receptors, some of them responding to submultiples of the frequency of stimulation. In their opinion it seems hard to believe that these discharges, so different from those resulting from steady stimulation of the stretch receptors, do not have a specific effect on the centers. The stretch receptors which subserve the appreciation of changes in posture seem thus to be concerned also in deep vibratory sensibility.

Applying vibratory stimuli to the teeth, Pfaffmann (29) found that the electric response from the dental nerves of the cat show a "frequency following" until an upper limit of 1500 cycles was reached. Since soft tissues of the skin yielded maximal values of 700 cycles under the same conditions, it was concluded that the higher values for the tooth result from the greater rigidity of the structures concerned. With a maintained stimulus, the amplitude of this response fell off together with a decrease in the accuracy of "frequency following." The major part of this diminution took place during the first few seconds of activity and the effect became progressively greater with higher frequencies. Studying the response from single fibers it was found that different fibers have different upper limits of "frequency following" (from 80 to 900 cycles). Endings with shorter adaptation times to maintained pressure in general seemed to display lower maximal frequencies, both to suddenly applied pressure and to the vibrating source, and to have higher thresholds. When stimulated at rates higher than their optimal frequency, the single fibers showed various types of fractional response, 1:2, 1:3, etc., which developed gradually at frequencies just supra-optimal, and more gradually at higher frequencies. The importance of these phenomena of synchronization for the interpretation of the events in the cochlear nerve is discussed.

PAIN

The problem concerning the afferent fibers responsible for the mediation of pain has in recent years been largely elucidated by electrophysiological investigations. It was first demonstrated by Adrian (2) that various noxious stimuli applied to the skin of the

frog set up a discharge characterized by the appearance of slowly conducted axon potentials, some apparently arising from fibers belonging to the *C* class. That afferent fibers belonging to this class take part in the production of nociceptive reactions was further strongly evidenced by Clark, Hughes & Gasser (8). They found that electric stimulation of the cat's saphenous nerve induced great effects upon respiration and blood pressure when all *A* and *B* fibers were blocked by asphyxia or pressure. They also drew attention to the close parallel in time relations between the disappearance of sensations in man during asphyxial nerve block, and the disappearance of the *A*, *B*, and *C* elevation in the electro-neurogram of the cat's saphenous nerve during a similar block.

The suggestion that the delayed second-pain response is due to slowly conducted impulses in *C* fibers (37) has been definitely proved by Lewis & Pochin (24). The delay is greatest at the most distal parts of the limbs. The rates of conduction obtained by measuring the reaction time to the second-pain sensation from different points of the limbs were found to agree closely with the rate of the *C* fibers. Pochin further demonstrates (30) that the delayed pain perception found in cases of *tabes dorsalis* is not due to an abnormal slowing of impulses in some diseased region, but to a defect in one group of pain fibers which conduct rapidly, revealing the effects of a group, which conducts slowly.

Noxious stimuli, which like radiating heat, do not make any deformation in the skin, elicit in the cat's saphenous nerve impulses only from slow δ fibers and *C* fibers (39). The after-discharge following upon a firm stroke upon the skin or after a needle prick is chiefly constituted by potentials from *C* fibers and there is a close parallel between the duration of the after-discharge and the burning after-sensation experienced in man. Recording the action potentials from dental nerves of the cat, Pfaffmann (28) finds that noxious stimuli of various kinds elicit potentials which from their spike height and configuration appear to be derived from fibers belonging to the δ group.

That pain is mediated both by a more rapidly conducting group of nerve fibers belonging to the δ group and by fibers of the very slowly conducting *C* class seems thus to be demonstrated beyond doubt.

In a recent paper on the theory of pain Achelis (1) emphasizes the importance of summation phenomena for the production of

pain. He quotes a number of observations which speak against the conception of a strict specificity of pain from the anatomical as well as from the functional point of view. Thus a pure pressure-spot sensibility loses its specificity when hyperalgesia appears, and the sensibility which is left when the pressure-spot sensibility is lost, involves as well as pain other sensations such as itching. That pure tactile sensations can be provoked by adequate stimuli from the conjunctiva and the cornea, which structures generally are believed to lack tactile nerve endings, is also taken as indicating that a strict specificity cannot be realized. Through the association of anatomical and functional conceptions of pain an attempt is made to find a new basis for a theory of pain. A release hypothesis ("Auslösungshypothese") of pain is advanced in which the relative independence of the peripheral and the central excitation sequences is placed in the foreground.

As was stated in the previous volume of this review, the conception that pain is mediated by specific nerve fibers seems, however, to be supported most widely. Much valuable information has also been gained from the study of the sensory dissociation produced by blocking the nerve fibers by different agencies. Rowbotham (32) reports that the human cornea must be supplied by specific tactile fibers. In two cases in which trigeminal tractotomy according to Sjöqvist's technique was carried out with precision, no pain could be provoked by any kind of stimulus applied to the cornea, but the patients appreciated the stimulus of a wisp of wool or the touch of a fine camel's-hair brush. Here the arguments that the sensation is dependent upon the mode of stimulation fail, since the dissociated anesthesia produced by the operation offers a ready anatomical solution. Lewis & Pochin (24) have reinvestigated the sensory losses due to asphyxia of a limb. The defects of touch, cold and warm sense, and rapidly conducted pain begin almost simultaneously. The defect in slowly conducted pain comes later and is preceded by an exaggerated pain response which is in accordance with previous observations. The only sense to be lost early is touch. Sensations of passive movement are lost early and fail at the same time as touch and deep pressure, while the sense of muscular tension is maintained longer. Pain in deep-seated tissues such as muscle is preserved until the very late state of asphyxia and until all sense of position is lost. These facts show that pain from muscles is mediated by specific fibers separate from

those conveying sense of position and local tension. This is in conformity with the observations of Echlin & Fessard (11) who presented evidence which strongly suggests that discharges of even very high frequency from stretch receptors are incapable of giving rise to a sensation of pain.

The correlation between the order in which nerve fibers are put out of action by different agencies and the rates at which they are capable of conducting, previously suggested to be significant, is imperfect according to Lewis & Pochin. That pain is mediated by fibers conducting at rates corresponding to the δ elevation and by fibers of the C class must be considered as definitely demonstrated. Similarly it is fairly obvious that temperature sensations are mediated by δ fibers (38), while tactile sensations are carried by β as well as C fibers (39). If the nerve fibers are put out of action by asphyxia according to the rates at which they are conducting, we should expect touch to be first affected, then temperature, "first pain," and finally "second pain." This is actually the case and, although Lewis & Pochin find a certain degree of overlapping, it seems difficult to deny that there is a fair correlation between the conduction rate and the susceptibility to asphyxia. Cooling superficial human nerves, however, blocks the fibers in an order which differs considerably from that produced by asphyxia and cocaine, according to Bickford (7). The sensation of cold is lost first, while warmth is the last sensation to be affected. Tickle is lost early when touch is becoming impaired. The cause of the different susceptibility of the different nerve fibers to these various agencies is as yet obscure.

Tickle and itching.—Cordotomy (section of the spinothalamic tract) in man, is accompanied by the loss of tickle on the analgesic side although tactile sensibility is intact or slightly impaired (12). Sjöqvist's operation (33)—sectioning of the spinal tract of the trigeminal nerve just below the olive, which, when adequately performed, leads to complete analgesia of the face—is also accompanied by the loss of tickle in the homolateral side of the face. A wisp of wool is reported as touch without any after-sensation (39). In the cat's saphenous nerve a similar stimulus sets up impulses in more rapidly conducting fibers as well as in C fibers and is followed by a weak after-discharge chiefly consisting of C potentials (39). Taken together with the fact mentioned above that tickle is lost early during asphyxia, tickle seems to be due to the summated

effect of impulses in touch and pain fibers. Further evidence for this view is provided by the observation that a tickling stimulus provokes pricking or slight burning sensations in cases of peripheral nerve lesions; the phenomenon may in some cases remain for many years. Further, after rubbing the skin properly, tickle cannot be produced for quite a while, although touch is unimpaired. Presumably the rubbing releases potassium ions in the skin which affect the superficial endings of the tiny *C* fibers, lowering their excitability.

Of great interest is a recent analysis of itching sensations by Bickford (6). Histamine punctured into the skin provokes after a certain interval spontaneous itching at the point of puncture. This is accompanied by a gradually developing change in the surrounding skin, which when gently rubbed gives an abnormal itching sensation. The former sensation is called "spontaneous itching," the latter "itchy skin." Evidence is advanced that itchy skin is produced by axon reflexes in nerve fibers in plexiform arrangement. These fibers are not sympathetic, as the itchy skin can be produced in skin deprived of its sympathetic nerve supply, and they seem to be separate from those which produce spreading hyperalgesia (nocifensor nerves). The fact that itchy skin is abolished by a degree of asphyxia which leaves spontaneous itching unaffected, indicates that the nerves mediating the two sensations are separate. As tickle also fails at an early state of asphyxia itchy skin is believed to be associated with tickle. After cordotomy itchy skin as well as spontaneous itching is lost, as might be expected.

Thus, while tickle and itchy skin sensations seem to be dependent upon the integrity of both the tactile and the pain-mediating pathways, spontaneous itching can obviously be produced by the pain fibers only. Introspectively, the after-sensation following upon a tickling stimulus would seem to be but quantitatively different from faint itching. Itching and burning after-sensation seem to fuse as the itching gets stronger, which would suggest that the difference can be attributed to quantitative changes in the discharge of a common group of fibers.

Hyperalgesia.—The assumption of a specific system of nerve fibers, the "nocifensor" nerves, was made by Lewis (20, 21) to account for the spread of hyperalgesia around an injured spot of the skin. Crushing the skin, and other noxious stimuli applied to the skin, give rise to a hyperalgesia which gradually spreads to become full in about ten to twenty minutes. It lasts for several or many

hours. The spread of the hyperalgesia corresponds closely to the cutaneous nerve which supplies the skin. Even when the injury is made on anesthetized skin the hyperalgesia appears as soon as the anesthesia recedes. The hyperalgesia can equally well be produced on skin which is deprived of its supply of sympathetic fibers, a fact which shows that the spreading hyperalgesia is produced by fibers belonging to the posterior root system.

The spread of hyperalgesia indicates that the fibers involved in producing the reaction are freely arborizing. As painful stimuli are very well localized in the skin, the conclusion is made that the hyperalgesia is not brought about by the fibers mediating pain. Lewis (22) has provided further evidence for this view, demonstrating that the nerve fibers concerned in spreading the hyperalgesia are blocked very early during asphyxia, while cocaine introduced into the skin paralyzes the pain nerves completely, and the touch fibers entirely, or almost entirely, before it interferes with the hyperalgesia reaction.

The nocifensor fibers thus, while not mediating any sensations, produce hyperalgesia and vascular reactions through the release of appropriate substances in the skin around the local injury. These effects are such as repel injury, guard against renewed injury, and aid local repair, hence the name "nocifensor." According to this working hypothesis of Lewis, hyperalgesia is produced by an entirely peripheral nervous mechanism. All evidence for the existence of the nocifensor fibers is, however, as yet of indirect nature.

An interesting observation was made by Lewis & Pochin (24) on a finger in which, as a result of long asphyxiation, a lasting condition of sensory loss with depressed tactile sensibility occurred. The hypoesthesia was associated with hyperalgesia. Warming the finger gave rise to increased hypoesthesia and exaggerated pain sensations, while cooling had the opposite effect. This effect of changing the temperature of the skin is reversible and occurs even in the absence of circulation.

That heat changes the excitability of the tactile receptors was previously reported by Zotterman (38, 40) from observations of the electric response in the lingual nerve and in branches of the saphenous nerve of the cat. The squirting of hot water upon the tongue, or on the skin, abolishes the response from the tactile receptors, leaving the activity of the pain fibers intact. The inactivation of the tactile receptors is only temporary; their excitability returns after cooling.

The association of hyperesthesia with hyperalgesia is a very striking phenomenon, occurring during asphyxia and after peripheral nerve lesions. According to Head's theory that epicritic fibers should exert an inhibitory action upon the protopathic system of fibers, the hyperalgesia in this case can be looked upon as a release phenomenon. Although many facts seem to speak much in favor of this view, the possibility cannot, however, be quite excluded that the hyperalgesia may be due to an increase in the excitability of the pain fiber endings occurring independently of the concomitant changes in the excitability of the tactile receptors.

Referred pain.—The manifold problems involved in the visceral pain mechanism have recently been reviewed by Stürup (34), who has made an extensive study of pain produced by distension of the esophagus in man. He reports that the appearance of pain is closely related to irregular contractions of the esophageal wall, particularly when these appear as a spasm. The pain is referred to the mid-parts of the chest and is accompanied by the appearance of an anemic and hyperalgesic zone in this part of the skin. Of particular interest is his observation that anesthesia of the hyperalgesic zone, to which the pain is referred, prevented the pain from being localized to this area, although the pain on the whole was not prevented. The usual "known" pain is either replaced by an unusual very poorly localized pain or is referred to parts of the skin outside of the usual area. The remarkable observation of Morley (26) that the shoulder-tip pain elicited by irritating the peritoneum under the diaphragm was abolished by anesthesia of the area to which the pain was referred, seemed to speak in favor for the old conception that viscerocutaneous reflexes are of great importance for the appearance of visceral pain. Woollard, Roberts & Carmichael (36), however, could not detect any effect of cutaneous anesthesia. Producing shoulder-tip pain by electrical stimulation of the phrenic nerve during phrenic avulsion, they found that anesthesia in no way affected the character or intensity of the referred pain and that the locality of the referred pain, apart from the occasional slight shift, was not altered. Hinsey & Phillips (17) also describe a case in which stimulation of the central part of the diaphragmatic peritoneum produced pain, which was regularly referred to a completely anesthetized area of the skin in the shoulder-tip region. In experiments on dogs and cats they found that the nociceptive sensibility produced by stimulation of the central portion of the diaphragmatic peritoneum is mediated en-

tirely by afferent fibers in the phrenic nerve, and that the thoracolumbar sympathetic pathways are not essential for the nociceptive reactions elicited, contrary to the opinion advanced by previous investigators (31).

That noxious stimuli applied to deep somatic structures produce pain which is referred, has been reported by Kellgren (18). Injection of hypertonic saline into muscle gives rise to diffuse pain while stimulation of fascia and tendon sheath gives sharply localized pain. The distribution of the diffuse pain from muscle appears to follow a spinal segmental pattern, which, however, differs from that of the segmental innervation of the skin. The referred pain from muscle is associated with referred tenderness of the deep structures. Further observations (19) showed that beneath the skin there is a second sensitive layer in which pain is localized with fair accuracy. This layer consists of the deep fascia, and such periosteum, ligaments, or tendon sheaths which are situated subcutaneously. All the deeper structures give rise to diffuse pain of more or less segmental distribution.

Lewis & Kellgren (23) have made a very thorough analysis of the pain produced by injection of hypertonic saline into the inter-spinous ligaments of man. This pain is very similar to pain experienced from visceral disturbances, displaying referred pain, superficial and deep tenderness, and tonic contractions of muscles of the trunk, the distribution of which is very similar or identical with those associated with visceral disease. In their opinion, deep somatic and certain visceral structures are supplied by a common set of pain nerves, stimulation of which produces similar pain and many similar reflex phenomena. From differences in the reflexes elicited by stimulating skin and deep-lying tissues they assume that the pain fibers to the skin belong to a system separate from those running to deep lying tissues. The cutaneous hyperalgesia appears to be identical whether provoked by visceral disease, by stimulating deep somatic structures, or by stimulating neighboring skin and is attributed to a common system of nocifensor nerves.

In order to elucidate the nature of the catabolite which is believed to cause ischemic pain from muscle, Maison (25) has determined the liminal concentration of potassium, ammonium, and sarcolactate ions injected into normal muscle and ischemic muscle which has been worked but is not painful. As none of the three limina were reduced by ischemic work of the muscle it is concluded that, if the culpable factor acts on nerve fibers in the intercellular

spaces, it is improbable that any one of the ions tested is the pain factor.

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PHYSIOLOGICAL PSYCHOLOGY

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Beyond a formal recognition of the philosophical problem of mind and body the chapter heading, Physiological Psychology, serves no purpose in what is to follow. In reviewing some of the many psychosomatic problems now being explored by physiologists, psychologists, and clinicians representing almost every medical specialty, no need arises for sorting out mental processes from various physiological functions observed in the same experimental situation.

PSYCHOBIOLOGICAL WITHDRAWAL IN SCHIZOPHRENIA

Recent studies on schizophrenia illustrate how little our traditional dualistic vocabulary need interfere with effective formulation and investigation of personality problems. The evidence shows that the lack of interest, apathy, and indifference of the schizophrenic patient—Cohen (1) refers to a "reduced, shrunken, defective organism"—are paralleled by physiologic hyporeactivity in general metabolism, functions of the autonomic nervous system, and functions of the central nervous system.

Cohen & Fierman (2) observed no ill effects from large doses of thyroid (15 to 18 gm.) administered daily for several months to eight schizophrenic patients. Despite an increase in oxygen consumption rate, the pulse rate tended eventually to decrease, and no excessive perspiration, tremor, or gastrointestinal disturbance was noted. Similarly, Freeman (3) found subnormal response in cutaneous temperature, insensible perspiration rate, and oxygen consumption rate after administration of dinitrophenol to such patients.

Freeman & Rodnick (4) found that their patients, upon breathing hot moist oxygen which blocked heat loss through the lungs, gave markedly less than normal responses in blood pressure, heart rate, and respiratory volume. Moreover, they exhibited little sign of discomfort in contrast to the normal controls where in many cases the reaction was so alarming that the experiment could not be completed.

Reduction of vestibular activity was demonstrated in schizophrenic patients by Angyal & Blackman (5). It was significantly reduced for rotary stimulation, and the difference in response between the patients and controls was even greater for caloric stimulation. In one patient alternating between stupor and periods which were relatively symptom-free, parallelism between vestibular reactivity and the clinical state was observed.

In an interpretive article Angyal, Freeman & Hoskins (6) suggest a significant and meaningful connection in the schizophrenic patient between psychologic withdrawal and physiologic withdrawal as illustrated by the instances of hyporeactivity just reviewed. They say,

In other words, it is possible that what is observed in schizophrenia is not resistance to thyroid medication, a lessened response to epinephrine, a diminished vestibular reactivity and psychologic indifference to stimuli of the physical and social environment, but only manifestations of one and the same condition. It may be useful to broaden the concept of withdrawal to designate not a purely psychologic but a "holistic" or "psychobiologic" phenomenon. . . . The type of approach we are suggesting here should not be a retreat into generalities. On the contrary, we wish to emphasize the necessity of specific and concrete studies. After a number of psychologic and physiologic features are brought together under the common concept of withdrawal, this concept, which is used at present with a somewhat vague and general connotation, gains a new and rather definite meaning. The phenomenon of withdrawal may thus be specifically defined as consisting of such and such psychologic and physiologic characteristics.

Since schizophrenic changes in reactivity vary from function to function and may even show increases, as in the blood pressure response to adrenal cortex (7) and in the increase of blood lipids after administration of testosterone, the authors suggest that the reduction of response may be most adequately expressed as a profile. From such a profile the investigator will be better equipped to devise a strategy of experimental attack upon the problem of the causation of schizophrenia.

PAIN AND HEADACHE

In reviewing the history and present status of the study of pain, Dallenbach (9) refers to three outstanding problems awaiting adequate solution, namely, identification of pain receptors, localization of the cerebral centers, and determination of the unique relationship between pain and unpleasantness.

Lewis (10) has urged revision of our language descriptive of

pain. Experiments have revealed the identity of the painful experiences derived from skin and mucous membrane as contrasted with the pain derived from deeper structures. So clear is this contrast that he thinks we may be in error in classing both together under the one term, pain. Simple tests are described for enabling a patient to differentiate clearly pain from skin, muscle, finger web, tendon, and periosteum and to compare the experimentally aroused experience with the spontaneous pain of which he complains. In this manner the patient may be briefly educated to describe his symptom accurately, thus aiding the physician's diagnosis.

Stone & Jenkins (11) have carefully reviewed the research of the past eight years on pain and temperature sensitivity. They discover "an excessive narrowness of approach and a deplorable lack of communication among investigators separated by language barriers or working in different compartments of scientific discipline."

It is possible, however, to select from the investigations of the past year a few which may be reviewed in orderly progression from the simple stimulus-response problem of pain threshold to the highly complex phenomena of headache involving, in the case of migraine, a detailed consideration of personality structure.

Hardy, Wolff & Goodell (12), following von Frey (1922) and Dallenbach & Stone (1934) in the use of thermal radiation, perfected a method for measuring the pain threshold. Light from a 1000-watt lamp focussed by a condensing lens on a blackened circular area of 3.5 sq. cm. on the forehead gave a clearly recognizable threshold pain (a sensation of heat, finally swelling to a distinct sharp stab of pain). Intensity of the radiation was then measured by placing a radiometer in the circular aperture in place of the forehead.

The measurements of pain threshold on three subjects over a period of a year did not vary consistently with time of day, general effectiveness, or emotional state. The deviation from the mean was not greater than ± 12 per cent. Heat and pain were distinguished by administration of acetylsalicylic acid which raised the threshold for pain but lowered that for heat. The peripheral structures responsible for pain were distinguished from those of temperature and touch by occluding blood flow to the hand for twenty-five minutes. Although the almost unbearable pain raised the pain threshold both on the ischemic hand and on the forehead as much

as 35 per cent, touch and heat sensation from the hand could hardly be elicited. Lack of spatial summation was also demonstrated and it was found that the ratio between the radiation evoking threshold pain and that causing tissue destruction (blistering) was about one to two.

A further study by Schumacher, Goodell, Hardy & Wolff (13) showed that differences in the reactions of individuals to pain are not the result of individual variations in pain threshold, which was relatively stable and uniform. In a group of 150 subjects of both sexes, under average conditions of well-being, 324 observations gave a pain threshold of 0.206 ± 0.03 gm. cal. per sec. per sq. cm. with a standard deviation for the group of ± 1 per cent. This threshold was independent of sex, was uniform throughout the 24-hour day despite varying moods and vicissitudes, and did not correlate with the subject's estimate of his sensitiveness to pain. To account for the variable distress experienced by different persons during similar pain stimuli it was necessary to distinguish between perception of pain (as determined by the pain threshold) and reaction to pain.

Wolff, Hardy & Goodell (14) emphasized the importance of this distinction in an analysis of the relation of morphine, codeine, and other opiates to the pain experience, employing the method described above for determining pain threshold. While opiates are known to lessen the distress experienced during pain, the analgesic action of morphine and codeine, as measured by rise in pain threshold, was reduced or obliterated by experimentally induced pain. This apparent contradiction disappears when it is realized that the complex psychobiological reaction to pain (including emotional, smooth muscle, gland, and skeletal muscle expressions) is independent of perception and can be dissociated from it. The authors observed that severe pain which was induced before administration of the opiate or early in its course of action reduced or neutralized its threshold-raising effect. They also found that when the blood pressure and pulse effects had disappeared following administration of epinephrine, although the subject still felt tense and excited, the threshold-raising effect of morphine was reduced or obliterated.

The psychological effects of the opiates were to free pain of its implications and to induce lethargy and sleep. While the peak of

the threshold-raising effect, regardless of the quantity of opiate given, was reached in ninety minutes these psychological effects were attained within twenty minutes and long outlasted the threshold-raising action. Moreover, when the threshold-raising action was obliterated or reduced by pain or epinephrine, the psychological effects such as "freedom from anxiety, contentment, and relaxation, though less pronounced, were still clearly evident." According to the authors, the pattern of withdrawal, flight, fight, and anxiety in response to pain is more stereotyped and predictable than the action patterns associated with perceptions of heat, sound, or cold, although dissociation of action from perception can be achieved in all these cases.

Similar instances of such dissociation of perception from reaction are observed in the experimentally developed conditioned reflexes of animals.

In this connection Whitehorn (15) has discussed the role of experiential conditioning in making possible the dissociation of the conventionalized patterns of behavior and internal symbolizations of "the emotions" from the "acute emotional experience," in which "the more raw or primitive facilities for biological adjustment are summoned up."

During operation in a series of patients Wolff (16) explored by faradic stimulation and by traction the sensitivity to pain of various structures in the head. Possible mechanisms for production of headache inferred from these observations included traction upon the veins of the longitudinal and other sinuses, on the middle meningeal artery, and on the large cerebral arteries at the base of the brain; dilatation and distention of the cranial arteries; inflammation of the dura and pia arteries at the base of the brain; and direct pressure or traction upon the fifth, ninth, and tenth cranial and upper cervical nerves. Penfield & McNaughton (17) have recorded observations on the innervation of the dura mater in man and monkey in connection with possible surgical procedures for relief from intolerable headache.

Reviewing the experimental studies on headache with particular reference to migraine—to which he and his colleagues have made important contributions (18, 19)—Wolff (20) has concluded that procedures which distend the relaxed walls of cranial arteries produce headache and that the headache of migraine arises chiefly

but not exclusively from distention of the branches of the external carotid. Ergotamine tartrate, by reducing the amplitude of pulsation in these arteries, will diminish or terminate the headache. Its intensity can also be diminished by digital pressure on the carotid artery of the affected side.

The hypothesis that the mechanism of migraine headache is similar to that of the so-called histamine headache suggested a recent investigation by von Storch (21). Since the ultimate cause of pain is in both cases vasodilation, he compared patients subject to migraine with sufferers from other chronic recurrent headache in their reaction to amounts of histamine too small to precipitate headache in subjects characteristically free from this complaint. The results showed that the threshold for histamine headache was lower in persons subject to chronic recurrent headache than in those not subject to this ailment and lower still in the case of patients afflicted with migraine. The histamine headache was frequently similar to the migraine headache but there was no actual attack with nausea and vomiting. However, it seldom resembled the habitual headache in those subject to other types of chronic recurrent headache. Finally, there was no evidence to show that there was any unilateral sensitivity of the vascular or nervous system to histamine in patients subject to hemicranial migraine headache.

Cranial tension was not interpreted as headache by the habitual sufferers, since those not conditioned by headache experience complained more bitterly than the others of the histamine headache. Other explanations of the lower histamine threshold, humoral and neurogenic, were suggested, and it was concluded that the results added another link to the chain of evidence connecting migraine and histamine headache.

According to Wolff (20) another problem concerning migraine is to determine those factors which throw the pain-producing mechanism into action. Since the attack is a relatively innocuous sign that the organism is under stress, the problem becomes one of determining the type of organism that reacts to stress in this particular way. The author has given a clear delineation of the rigid personality structure found in the migraine patient and has stressed the importance of viewing the life experiences in terms of a long time span if one is to understand the relation of the attacks to personality features, emotional reactions, and situations.

PSYCHOBIOLOGICAL ASPECTS OF PHYSIOLOGICAL FUNCTIONS

Techniques for measuring various physiological functions in relation to the play of thought and feeling in the human subject have recently been widely employed in psychosomatic investigations. Such studies are directed toward the same goal which Pavlov set for himself in his work on the highest nervous activity. In a recent critical review of experimental investigations concerning animal and human conditioning Hilgard & Marquis (22) have considered "operant" behavior, problem solving, voluntary action, and personality in relation to conditioning experiments of the classical Pavlovian type. A chapter devoted to the history of research on conditioned reflexes will aid the reader in gaining more than a formal acquaintance with the scope and aims of this field of experimental psychobiology.

Salivation.—Following the earlier experiments of Krasnogorski, and Winsor, Finesinger and Strongin, with their collaborators, studied the parotid salivary responses in normal subjects and in mentally disordered patients. These investigations constitute a legitimate extension of Pavlov's experimentation on the salivary conditioned reflexes of the dog.

Finesinger & Sutherland (23) studied salivary conditioned reflexes in fifteen psychoneurotic patients and five control subjects. Positive and negative reflexes were readily established and the usual phenomena of conditioned reflex action found in animals were observed, including extinction and internal and external inhibition. Lemon juice was employed as the unconditioned stimulus. During periods of disturbance in the psychoneurotics pronounced deviations toward excitation or inhibition were observed. Episodes of excitation (or "irritation") were characterized by marked increase in secretion during the periods between successive stimuli. Inhibition showed itself by increased latency and diminution or disappearance of the positive reflex. In extreme inhibition, increase in the latent period and diminution of secretion in response to the lemon juice were observed. The secretion occurring between successive stimuli (interval secretion) seemed to give the most reliable indication of disturbed emotional state.

Dispensing with conditioned and unconditioned stimuli Strongin & Hinsie (24) relied on measurements in patients with mental disorders of the continuous parotid secretion in the absence of ex-

terostimulation. Over a two-hour period the secretion in normal subjects ranged from an average of 0.02 to 0.15 cc. in five minutes. In five schizophrenics, where deterioration had already set in and the prognosis was very bad, the secretion level was above the normal, ranging from 0.23 to 0.74 cc. in five minutes. In six manic-depressive patients (25) during the manic phase the secretory rate was within normal limits while during the depressed phase it ranged from 0.001 to 0.01 cc. in five minutes, a rate definitely below the normal. Preliminary results, difficult to interpret, were obtained by Strongin, Hinsie & Harris (26) in ten patients diagnosed as dementia praecox, when the level of parotid secretion was determined before, during, and after insulin hypoglycemic therapy. One definite result was that the secretory level during the hypoglycemic state showed a sharp rise with the onset of stupor and coma. However, sleep after insulin administration lowered the secretory level.

These promising parallel studies of Finesinger and Strongin suggest the desirability of further experiments comparing the two techniques since both seem to yield significant data concerning salivary secretion in relation to the patients' mental status.

Gastrointestinal function.—Eisenbud (27) tested the hypothesis that the function of repression varies directly with parasympathetic excitement, employing as his subject an eighteen-year-old girl, with "epileptoid personality." He recorded gastric motility and secretory rate of the parotid when, under hypnosis, the subject was caused to live through a trumped-up emotional experience and commanded to forget before being awakened. This posthypnotic amnesia introduced a factor which he believed to be "tantamount to repression." The observed increases in gastric motility on awakening, sometimes preceded by a brief period of inhibition, harmonized with the above hypothesis. Increases in parotid secretory rate, which are suggestive in the light of the findings of Finesinger & Sutherland, require more careful controls than he found possible at the time.

Mittelman & Wolff (28) discussed emotionally charged life situations with nine patients suffering from gastric neurosis and peptic ulcer. During the affective states thus induced, gastric motility increased and acidity values rose, sometimes after an initial fall. In patients with peptic ulcers, blood occasionally appeared in the stomach content. Similar changes appeared during sleep when the patient had just previously been subjected to affective stress.

In a recent review, Brush (29) emphasized the importance of emotional difficulties as basic or contributing factors to the increasing incidence of gastrointestinal disorders. White, Cobb & Jones (30), analyzing sixty cases of mucous colitis, have supported the thesis that tensional states of long duration "lead to chronic stimulation of the autonomic centers and in certain instances to the liberation of acetylcholine in the parasympathetic endings in the colon. Symptoms arise when an unfortunate combination of psychological and physiological events cause morbid functions." In the cases reviewed the patients exhibited characteristics of the personality consistent with chronic tension, such as overconscientiousness, dependence upon the opinions of others, and sensitivity.

Respiration.—In discussing his investigation of salivation Fine-singer (23) contrasted it with a similar study of respiratory tracings. He considered the salivary record as more reliable than the respiratory tracings because in salivation voluntary control is at a minimum and in the case of respiration the tracings are more difficult to evaluate. This practical judgment was based on the results of a painstaking study of the effect of pleasant and unpleasant ideas on respiration in forty-one psychoneurotic patients and twenty-one control subjects (31). A review of the literature beginning with Mosso in 1880 (59) indicated that previous conflicting results were due mainly to the small number of subjects employed in the earlier experiments.

The psychoneurotics were classified in two groups. The twenty-nine patients in group 1, whose symptoms were diagnosed as hysteria, phobia, or anxiety neurosis, reacted as a group with pronounced respiratory changes when directed during the experimental period lasting a quarter to half an hour to think of pleasant, of unpleasant, and again of pleasant ideas. The twelve patients in group 2, whose conditions were diagnosed as hypochondriasis, reactive depression, or compulsion neurosis, reacted with very slight or frequently with no respiratory changes. The normal subjects reacted less than the patients of group 1 and more than the patients of group 2.

The respiratory tracings were analyzed for the five periods of each experiment for rate, depth, minute respiratory volume, metabolic rate, and spread of the expiratory-inspiratory angle.

The most consistent changes were observed for the minute respiratory volume. Between 86 and 96 per cent of the patients in group 1 and of the controls showed a

considerable increase in minute respiratory volume for the unpleasant period as compared with the two pleasant periods, and for the same comparisons over 75 per cent of the patients in group 2 showed a very slight increase in minute respiratory volume. About 90 per cent of patients in group 1 and of the controls had a higher minute respiratory volume during the unpleasant than during the preliminary period. For this comparison no consistent change was shown by patients in group 2. All the patients in group 1 and over 90 per cent of the controls showed a marked decrease in minute respiratory volume during the relaxed as compared with the unpleasant period.

Finesinger speculates concerning the cause of the lack of respiratory reactivity of the patients whose conditions were diagnosed as hypochondriasis, reactive depression, or compulsion neurosis (group 2). It is possible that their lack of reactivity was due either to a relative lack of affect, or to some consistent predominating mood which could not be shifted by the suggestions of the investigator. He thinks it might be fruitful to determine whether the lack of reactivity observed for the respiratory responses holds also for cardiac and other types of response. He suggests that from the standpoint of responses involving the autonomic nervous system it is possible that these patients react more like psychotics than like the psychoneurotics in group 1 of his study. This would be of interest in regard to Freud's theory relating hypochondriasis dynamically to the psychoses.

In a further investigation in psychoneurotic patients and normal subjects, Finesinger & Mazick (32) employed changes in minute respiratory volume as an index of respiratory response to a painful stimulus and its recall. The results of their previous study were confirmed, the response to a painful stimulus and its recall closely paralleling the response to an unpleasant ideational stimulus.

Two other studies of respiration in relation to psychological processes emphasize a different aspect of the problem. Sutherland, Wolf & Kennedy (33) report briefly on the respiratory curves drawn by patients suffering from neuroses and psychoses. They employed the recording spirometer used in determining basal metabolic rate. The subject was required to breathe naturally into the spirometer for about a minute and was then asked to inspire deeply to breathe naturally again, to exhale deeply, to breathe normally, and finally to hold the breath as long as possible, normal respiration this time being followed by hyperventilation.

In several hundred abnormal records, marked variations from

the normal were observed, less in neurosis than in psychosis. Records from the same patient taken three to twenty-eight days apart reproduced each other with some precision. When duplicate records which had been taken days apart in several hundred cases, were shuffled, they could be paired quite easily. Consequently the authors characterize these spirometric pictures as respiratory fingerprints of the nervous state of their patients. As the patient improved clinically his spiropoint approached the normal, or if his mental state became worse, his respiratory tracing became more irregular and bizarre. In spite of these changes in the mental state the patient's "respiratory personality shines through" and is easily identifiable as his own.

Alexander & Saul (34) were similarly preoccupied with the individuality of their patients' respiratory tracings. Their description of respiratory curves in a preliminary report on respiration and personality is the first step in a planned research based upon logical expectation. They sought to discover whether the psychological tendencies with incorporating, eliminating, and retaining vectors that they had found in previous work could be brought into causal relationship with certain gastrointestinal disturbances, and whether they might not have an influence upon the respiratory act where all three of these vectors find expression ("incorporating tendencies in the inspiratory act, eliminating tendencies in the expiratory act and retentive in breath holding").

Using an ordinary metabolism apparatus with very low resistance to respiration and enlarged to permit inclusion of the vital capacity on the same tracing, they considered for detailed description the following: rate; depth; respiratory level; rounding of tips, hooks, and squares caused by breath holding; and spikes. Their subjects included groups of asthmatics, hebephrenics, and paranoids. No correlations with chest shape were found, but in agreement with Sutherland, Wolf & Kennedy they noted that the spirogram is typical of the individual, like his handwriting, no two individuals yielding identical tracings. In about three fourths of their cases the major features of the tracings remained characteristic of the individual over periods as long as three years.

Asthma.—McDermott & Cobb (35) studied fifty cases of bronchial asthma which were taken without selection from an allergy clinic. Only young children were excluded. Medical records were examined and about two hours were spent with each patient. In

this psychiatric study thirty-seven of the fifty cases revealed an emotional component in their asthmatic attacks. Neurotic traits other than asthmatic appeared in thirty patients. Somatic therapy benefited only 20 per cent of the "emotional" group while 54 per cent of the "non-emotional" group (predominantly young males) were benefited. Benefit was derived from drugs and biological products by 20 per cent of the patients in the neurotic group but by 50 per cent of the nonneurotic patients.

In continuation of the above study a few cases of bronchial asthma were selected for psychoanalysis by Deutsch (36). He reviewed in detail the psychosomatic relations encountered in three of the patients who had been treated unsuccessfully for many years. The typical psychological structure of female asthmatics was found to be derived from an affection of the respiratory apparatus in early childhood which coincided with certain emotional factors; these developed from temper-tantrum behavior during childhood, from great dependence on a domineering and aggressive mother (combined with hatred), and from attachment to an easy-going father. In the course of psychoanalysis asthmatic attacks could be provoked by stimulation of the latent hatred of the mother or could be suppressed by dissolving the psychological constellation provoking this hatred. Asthma disappeared if the emotional situation did not contain the specific associative material necessary to precipitate an attack. But asthma recurred if this specific psychological stimulus was in any manner created, e.g., by a visit from the mother.

Vasomotor function.—Three years ago Menzies (37), in an extensive series of experiments, demonstrated a variety of conditioned vasomotor responses in human subjects. Unconditioned vasoconstriction was excited by immersing one hand in ice water. A thermopile and high sensitivity galvanometer provided continuous records of the skin temperature of the other hand. In twelve out of fourteen subjects, stable conditioned responses were established by nine to thirty-six combinations of signal and cold stimulus. The conditioned stimuli included a pattern of light and the sound of buzzer and bell. Conditioning was established when a nonsense word was whispered by the subject or when the word was both pronounced aloud by the experimenter and whispered by the subject. Although conditioned responses were elicited more frequently by bell or buzzer, they tended to be less permanent than the verbal

conditioned reactions. Marked individual differences were observed in the changes of skin temperature accompanying excitement, anxiety, affectively toned thinking, drowsiness, mild fatigue, and protracted fixation of attention.

These mental states in relation to skin-temperature changes were the object of a carefully planned investigation by Mittelman & Wolff (38). Employing Hardy's radiometer (cf. 12) they made 203 observations on forty-seven subjects. In all but three instances a drop in the skin temperature of the fingers occurred under emotional stress; these instances were confined to two subjects. Affective states were induced experimentally by a discussion dwelling on difficulties in the individual's life situation to which he reacted with signs of distressing emotion. In some cases tasks were required, such as the memorizing of digits with repetition forward and backward.

In these experiments the physical environment was carefully controlled as to temperature and humidity, and the subject, who was lying lightly covered, was urged to relax. Under these circumstances skin temperature of the fingers remained fairly constant (between 31°C. and 36°C.) during the control period. With the affective stress aroused during the interview the temperature of the fingers on some occasions fell below room temperature. In an anxious subject in a cool environment (21°C.) finger temperature reached a high level very slowly in contrast to the prompt rise when the environment was warmer. Moreover, in the cool environment the drop in temperature of the fingers during experimentally induced stress was greater than that observed in the warm environment.

In cases of Reynaud's disease, the amount of pain or incapacity suffered by the patient was shown to depend upon the interplay of affective and physical environmental influences which counteracted or reinforced each other in their effect on the circulation of the extremities. Wide individual differences were shown in the fall of finger temperature under the influence of distressing emotion aroused by the interview. In two cases, for example, a discussion of the patient's baffling personal problems produced no greater fall in finger temperature than the task of memorizing digits. In another case a high school girl, reading an address by President Roosevelt at the dedication of a medical center, experienced anxiety, insecurity, anger, and resentment. She said, "Two of my brothers

are working on a government project, being supported by the government. They will vote against Roosevelt in the coming election. That's not fair to him. We have had many quarrels about it at home."

Ovarian function.—Landis and his colleagues (39) have investigated the growth and development of the emotional and sexual aspects of personality in 153 normal women and 142 female psychiatric patients by means of controlled interviews consisting of "questions concerning the facts and phantasies related to psychosexual development." Additional techniques of investigation included a marriage inventory, general information inventory, physical examination, vocational interest blank for women, roentgenological studies of the pelvis, and case histories of the psychiatric patients. The authors have not tried to prove anything but have presented facts and their relationships to serve as baseline data for future investigations in this field of psychobiology.

Benedek & Rubenstein (40) have reported correlations between ovarian activity and psychological processes in adult women. Their study of the ovulative phase of the menstrual cycle was based upon seventy-five cycles of nine neurotic patients of child-bearing age. All but twenty-three of the cycles were anovulatory. Novak (41) calls attention to the fact that failure of ovulation occurs not infrequently in ostensibly normal women. In the experiments of Benedek & Rubenstein (42) the data concerning the premenstrual-menstrual phase were obtained from the observation of 125 cycles of fifteen patients. In the preliminary period of the investigation the daily vaginal smears and records of rectal temperature were sent to Rubenstein in Cleveland while the psychoanalytic records were studied by Benedek in Chicago. After ten months the investigators met to compare their data and found an exact correspondence of the ovulative dates as independently determined. During the whole period of study the endocrine and psychoanalytic records were independently organized and evaluated, and were then compared.

Rubenstein (43) had earlier shown that body temperature and basal metabolic rate seem to be equivalent measures of ovarian activity. Basal metabolic rate fluctuates widely but regularly during the menstrual cycle, lowest values occurring about the thirteenth day of a 28-day cycle. Body temperature varies in the same way,

the low point coinciding with the more highly cornified (follicular) vaginal smear.

It was found to be easiest to make quantitative estimates of the intensity of instinctual drives in neurotic women whose inhibited drives call for strong defense reactions such as "noisy" masculine protest or anxiety against heterosexual desire. The following correlations were obtained. Whenever evidence of heterosexual drive appeared, the presence of estrone was inferred from the character of the vaginal smear. The erotization of the female body dominating the psychoanalytic material was accompanied by evidence of progesterone activity derived from interpretation of the smears), while an abrupt decrease in heterosexual tension, together with an influx of passive libido tendencies, accompanied ovulation as determined by the vaginal smear examinations and basal body temperature determinations.

During the premenstrual-menstrual phase the correlations were more difficult to establish because, with low gonadal function, the instinctual tendencies which the hormones control were not clearly revealed in the psychological material. However, the knowledge gained from the much clearer preovulative-ovulative phase of the cycle made possible the prediction of the smear interpretation from the analytic material. The premenstrual-menstrual phase was inferred to be one of diminishing progesterone and low, but variable, estrone production with the metabolic rate generally decreasing. The instinctual tendencies were believed to be on the genital level with relatively high hormone production and on the pregenital level with very low hormone production, eliminative tendencies appearing upon extinction of progesterone. However, the emotional reactions during this phase were more intense and complex than could be explained on the basis of the low hormone level inferred. Emotional relaxation was observed to accompany the establishment of menstrual flow.

The authors suggested the possibility that psychological factors influence gonad function. However, the evidence shed no light on the problem of primacy of gonad or psyche. In reviewing the relation of the nervous system to the physiology of the female reproductive system, Hinsey (44) says, "Meager as our knowledge may be, we should ever be cognizant that in some way emotional states influence certain phenomena of the female reproductive tract. . . .

Whether this is to be attributed to a nervous effect on the anterior lobe, the ovary, the uterus itself, or all of them is an enigma."

Since Papanicolaou's (45) studies of vaginal smears in normal women, improvements in the technique have been made. Shorr, in two recent notes (46, 47), has presented the advantages of a modification of the Masson trichrome stain. This stain makes possible specific and reliable detection of cornification. A sequence of contrasting color changes resembles a chemical titration in sharpness, contributing greatly to the ease and certainty of the interpretation of the smear. Later, he revised the technique for the use of domestic stains exclusively, simplifying and shortening the procedure previously described. The vaginal smears of the first half of the menstrual cycle are, according to Shorr (48) more clearly defined and best understood, since the epithelium and secretion are largely, but not entirely, under the control of the estrogenic hormones. The changes in the smear during the second half, from the time of ovulation up to the next menstruation are less clear-cut and more difficult to evaluate. Here the structure of the vaginal epithelium is a resultant of the interaction of estrogenic and progestational hormones. Variations in the tempo or extent of their production could be expected to determine the smear picture.

Menopause.—In nine women with spontaneous menopause or menopausal syndrome, a follicular type of vaginal smear was induced by administration of estrogen. When this stage was reached, estrogen treatment was continued along with varying dosage of progesterone. The combined therapy induced virtually all of the changes seen during the second half of the normal menstrual cycle. Psychological changes accompanying the administration of these hormones were not reported.

In considering the problems of the menopause Ripley, Shorr & Papanicolaou (49) tested the effect of estrogenic hormone in a group of menopausal and postmenopausal depressions, including cases of involutional depression, depression of the manic-depressive type, and depressive illnesses of a more reactive nature where inherited tendencies as well as psychoneurotic factors were present. The ages ranged from thirty-nine to fifty-eight years. The depressive illness as such was not influenced specifically or its course shortened, but relief of vasomotor symptoms with improvement in feelings of well-being was observed. It was found that there was

considerable variation in the amount of estrogenic hormone necessary for the development of the follicular type of vaginal smear.

A further study by Shorr (50) showed that the "human" unit for full replacement—that is, the development of the follicular type of smear in patients exhibiting the menopausal syndrome—lay between 1,000 and 10,000 rat units daily. Wide variations from patient to patient in the subjective and biological response to estrogens made any attempt at standardization of dosage futile. Shorr believes that the menopausal syndrome is a symptom of a more fundamental psychological maladjustment, that is, failure to adjust to the new internal environment which follows the cessation of ovarian function. Why it occurs in certain women and not in others is unknown. Shorr says,

The anticipation that the estrogens would function as sex hormones in the sense that they would be able specifically to influence libido, has not been realized. There is a growing awareness that the sexual drive is grounded in more fundamental factors and that the concept that it was solely dependent on the reproductive secretions was an oversimplification of an extremely complex and subtle reaction.

Other methods for psychobiological study of ovarian function.—In 1938 Burr & Musselman (51) reported correlation of the menstrual cycle in women with differences in potential of the index fingers. They found that marked voltage rises were usually observed once in each cycle. Some cycles occurred without the appearance of such marked increases in potential differences.

Barton (52) made over 200,000 determinations on seventy-nine women and 238 menstrual cycles. From this study of the electrical correlates of the menstrual cycle she found that, although significant peaks occur more frequently in the mid-period, they may be found in every decile of the menstrual cycle, even during menses, and more than one peak may occur in a cycle, while in long menstrual-cycle studies significant peaks may spread over many deciles. The marked rises in voltage do not appear in menopause, sterility, or male records. From the evidence presented it is inferred that these peaks in index finger potentials record the time of ovulation.

With the development of such precise methods for following the course of the estrous cycle in animals and man, new possibilities appear for the investigation of the detailed psychobiological phenomena related to the action of the female sex hormones. Ben-

edek & Rubenstein have shown the possibilities of psychoanalytic procedure in discovering the specific correlations between hormone production and instinctual drives, while Stone (53) has described reliable methods which have been employed in the study of the sex drive in animals.

Altman (54, 55) has studied the behavior of the sow in relation to its sex cycle, which varies from eighteen to twenty-three days. Behavior as determined by spontaneous activity, and conditioned salivary and motor reflexes was compared from day to day with the stages of the estrous cycle as indicated by vaginal smear, nasal smear, steady state potentials from the skin above the ovaries, and rectal temperature. Results showed that the phases of the cycle were most adequately recognized by the observation of external signs of heat, spontaneous activity, and steady state potentials, while the vaginal smear, nasal smear, and rectal temperature did not indicate clear cyclic differences. Significant changes at estrus were observed in decreased vigor of motor and salivary conditioned reflexes, increase in spontaneous activity, and friendlier disposition toward the experimenter.

In reporting on the management of sexual activities by the major levels of central integration Bard (56) says,

The available evidence indicates that estrin exerts its specific effect on the central nervous system at some level above the lower portion of the mesencephalon,

and concludes,

It has been demonstrated that in several species of mammals the full pattern of mating behavior can be elicited after removal of all cerebral cortex. When under the influence of ovarian hormones, wholly decorticate female cats, rabbits, and guinea pigs exhibit typical estrual behavior. After removal of all cortex male rabbits are able to execute effective copulatory behavior. These patterns of sexual response must therefore be elaborated by subcortical mechanisms. There can be no doubt, however, that in any mammal the presence of the cerebral cortex greatly increases the number of circumstances which are able to modify the occurrence of sexual behavior.

PSYCHOBIOLOGICAL PATTERN IN RELATION TO PHYSICAL AND CULTURAL ENVIRONMENT

Sleep.—Kleitman (57) has proposed an evolutionary theory of sleep. "Wakefulness of necessity" is a subcortical and probably hypothalamic function. The wakefulness center is aroused in the newborn baby or decorticate dog only in response to hunger, wet, cold,

or other such disturbance. After primitive need has been satisfied or external irritation removed, the individual again falls asleep. The supplementary "wakefulness of choice," as well as the diurnal sleep-wakefulness cycle, is a cortical function. Unlike the female sex cycle, sleep and waking follow a conditioned 24-hour cycle because babies are born into a world run on a 24-hour schedule. It was found that some experimental subjects could become adapted to a 21- or 28-hour period within a few days.

Between frank wakefulness of necessity and definite wakefulness of choice there are many gradations, dependent upon the greater part played by the cerebral cortex in the animal's activity, in the course of phylogenetic and ontogenetic development. Therein lies the evolutionary feature of this theory of sleep and wakefulness. Pure wakefulness of necessity, where it exists, is characterized not only by an absence of pleasure but by its appearance of being forced on the animal corresponding to what Ranson calls the hypothalamic drive.

In his recent book Kleitman gives a comprehensive and meticulous account of experimental investigations concerned with all aspects of the sleep-wakefulness cycle, including his own extensive researches since 1923. Thirteen of the thirty-six chapters include the results of investigations not hitherto published, for which Kleitman acknowledges the coauthorship of fifteen of his collaborators. The new researches include studies of electrical skin resistance, blood composition and blood volume, variation in intracranial pressure in wakefulness and sleep, diurnal temperature and motility curves in the feeble-minded, diurnal variations in blocking, diurnal curve of phosphate excretion, modifiability of the diurnal temperature and motility cycle, seasonal variation in motility during sleep, motility during sleep in psychopathic and mentally retarded individuals, and the effects of amphetamine on blocking and on the ability to stay awake. The mere listing of these items of recent research completed in one laboratory in the course of the planned investigation of a physiologist interested in the problem of sleep illustrates the futility of attempting to label the various manifestations of sleeping and waking as either psychological or physiological.

Convalescence.—When the boundaries of observation in psychology are extended beyond the limits set by the techniques of laboratory experiment or controlled interview many fundamental problems present themselves. For example, convalescence involves a multitude of problems the solution of which must be sought, not

only in clinical laboratories, but in social and economic conditions found in the homes of patients seeking to regain health.

In a recent symposium on convalescent care (58) Pepper emphasized the persistent deviations from the normal in metabolism, body chemistry, and physiology during this critical period and directed attention to the need for specific and individualized knowledge concerning the results of each disease, of surgery, of trauma, and of emotional shock. Canby Robinson discussed the convalescent patient as a person, that is, "an individual with a social status and a conception of the role he desires to play in the group." The importance of emotional factors and personality adjustment was stressed, particularly in the convalescent care of aged patients (Barker), cardiac patients (Levy), and patients recovering from thyroid operations (Colp).

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ANATOMICAL STUDIES

Afferent arterioles and juxtaglomerular cells.—Studies of a large series of vertebrates show that both the macula densa or epithelial plaque, and the polkissen or periarteriolar pad are absent in reptiles and fish; that the plaque alone is present in the frog; and that the polkissen are poorly developed in birds (67). The percentage of glomeruli with epithelial plaques varies from 50 in the frog and bird to 95 to 99 in the rabbit, man, and whale. The juxtaglomerular cell groups are not found in the kidneys of children under the age of two (157). In young adults, the wall of the afferent arteriole may show all transitions from an elastic type to an epithelioid or afibrillar cell type (9, 51). Marked hyperplasia of the afibrillar cells in the arteriolar wall and of the juxtaglomerular cells follows shortly after partial clamping of the renal artery in dogs (104) and rabbits (66, 102, 103); in rabbits numerous granular cells develop from afibrillar ones in a manner strongly suggestive of an endocrine secretory function, presumably concerned with the production of the renal pressor principle and the regulation of normal renal arteriolar tonus. There are no granular cells in the arteriolar media of the normal rabbit kidney. The ischemic kidney shows mitoses only in the afibrillar cells, which are apparently stem cells for the secretory granular cells (66). Water diuresis in the mouse is reported to be associated with a decrease in the number of visible polkissen, and pituitrin oliguria with an increase (9). Mercury poisoning has no effect upon the juxtaglomerular cell group (67). The significance of hyperplastic afibrillar cells in the human hypertensive kidney cannot be evaluated until a larger series of control kidneys has been studied (110). A critical review of this phase of renal anatomy has been written by Smith (247).

Tubules.—The origin of the juxtaglomerular cells has been ascribed to a pinching-off of epithelial cells from the ascending limb of Henle's loop and the distal convoluted tubule (81). The

¹ This review covers developments during the two years since the publication of the first volume of the *Review*.

function of these cells may be to secrete something which can cause the adjacent arteriolar afibrillar, or epithelioid, cells to swell or shrink and so regulate the flow of blood into the glomerulus.

The number of "special cells," demonstrable by silver impregnation in the renal medulla of the rat, varies directly with the amount of chloride reabsorbed in the distal tubule and inversely with the serum chloride level (80).

Large doses of tryparsamide injected into rats cause extensive necrosis of the distal convoluted tubules and less necrosis of the proximal tubules, followed in both cases by the usual repair. Tolerance can be established, but in contrast to other examples of resistant renal epithelium (41), the tryparsamide-fast cells are indistinguishable from normal cells (180).

Interesting reconstruction studies of normal and diseased tubules have been reported (199, 261).

Glomeruli and blood flow.—Supravital staining and vascular injection of the kidneys of the dog, cat, rabbit, guinea pig, and rat demonstrate that the original normal mammalian renal blood supply is exclusively glomerular and that the 5 per cent of variant glomerular units, including Ludwig's arteriole, represent pathological deviations acquired by the adult animal as part of the vicissitudes of aging (179). The degenerative processes that convert the glomerulus either into an obliterated nonfunctional, nonvascular unit or into a single patent vessel, an arteriola recta vera, are well illustrated. The possibility of nonglomerular blood flow in human disease is emphasized in recent anatomical (68) and physiological (247) studies. However, there is no good evidence for the existence of functional arteriovenous shunts in the normal mammalian kidney (255).

Glomerular functional intermittence is not evident in dogs and rabbits whose renal arteries have been injected with India ink (284). Apparent intermittence can be explained by the chance failure of the ink to enter a preglomerular or large artery. Intravital microscopy of frog kidneys after the injection of fluorescein and acriflavin reveals little or no spontaneous glomerular intermittence (74).

During the embryonic and early postnatal period the human glomerulus has fused capillary loops and a high epithelium, thus being poorly equipped for filtration (119). The inulin clearance is subnormal in very young infants (13).

GLOMERULAR FILTRATION

Comparison of inulin and creatinine clearances in certain pathological states leads to the conclusion that creatinine can diffuse back through damaged tubules and so lose some of its usefulness as an accurate measure of glomerular filtration. Thus, in uranium poisoning in the dog, the ratio of creatinine to inulin clearances falls to 0.76 or less, with a low extraction ratio (128). Similarly, the pump-lung-kidney preparation develops a falling creatinine to inulin clearance ratio as the ratio of urine to plasma inulin rises; the completeness of water reabsorption in the absence of osmotic limitation from urinary chloride is the important factor (242). Chloralose anesthesia in the dog does not affect the clearance ratio. If the plasma concentrations of creatinine, inulin, ferrocyanide, and xylose are kept constant, and the blood pressure and urine flow are unchanged, the clearance ratios are the same for the pump-lung-kidney preparation as for the explanted kidney (130). Low ratios of creatinine to inulin clearances have been found in human diabetic coma with hypotonic urine containing very little glucose or chloride in spite of high serum levels (189). Unfortunately, no histological studies are available on this interesting "functional disorganization" of the kidney.

The inulin clearance can be accurately estimated at plasma inulin levels below 20 mg. per cent (194). No tubular reabsorption nor excretion of inulin is apparent at a plasma level of 5 mg. per cent. The glomerular filtration in infants, estimated simply from the slope of the falling plasma inulin curve, is only 20 to 40 per cent of normal in four- to nine-day-old infants (13), this inefficiency correlating with the anatomical immaturity of the glomeruli (119).

Water diuresis increases the inulin clearance in the chicken, as does epinephrine without inducing diuresis (170). Epinephrine polyuria occurs in the glomerular puffer but not in the glomerular toadfish (273).

The ratio of endogenous creatinine to inulin clearances may rise to 1.73 in patients with very low renal function, suggesting decreased glomerular permeability to inulin or secretion of endogenous creatinine by hypertrophied tubules (259). In view of the variable results obtained with different methods of analyzing for creatinine-like chromogens in human plasma, no reliable conclu-

sions can be drawn from endogenous creatinine clearances in man (252).

Excess of vitamin A increases the inulin clearance in the dog, while avitaminosis A diminishes it about 50 per cent; other vitamins have no influence upon the glomerular filtration rate (134).

From a critical consideration of extensive experimental data on normal man as well as from theoretical equations expressing the hydrostatic factors involved in filtration under varying conditions of renal blood flow, a concept of glomerular dynamics has been evolved in which efferent arteriolar tonus is the chief local regulator of renal blood flow under conditions of constant glomerular filtration and approximate filtration equilibrium (249). (In renal disease glomerular dynamics may well be altered by changes in afferent arteriolar tonus, by conditioned permeability of a thickened glomerular membrane, and by other factors.) Whether the parallelism of filtration and renal blood flow in the dog, in contrast to the constancy of filtration in man, represents a species difference (as in the case of creatinine and ferrocyanide clearances) or is the result of different experimental conditions, has not been determined (274).

TUBULAR EXCRETION AND REABSORPTION

A fundamental review of this subject (241) distinguishes clearly between tubular excretion, reabsorption, and synthesis, and analyzes the mechanism of excretion and reabsorption as follows: (a) transfer of solutes is determined by organization of the tubule cells and thermodynamic principles; (b) there is present in the tubular epithelium of all species a limited amount of a stable cellular element whose specific function it is to combine temporarily with the solute transferred; (c) quantitative limitation to the transfer of solutes in either direction is set by the concentration of the solute in the peritubular fluid or tubular lumen. In all cases of tubular excretion the rate of transfer is curvilinear, reaching some constant maximum; however, several solutes—such as phenol red, diodrast, and hippuran—may compete for the same cellular mechanism and interfere with each other's maximal excretion quantitatively. The maximal rate of tubular excretion of diodrast, the diodrast Tm , may be considered as a reliable indirect measure of kidney size because there is a high correlation between the plasma diodrast and inulin clearances, on the one hand, and the diodrast

T_m on the other (99). Comparison of the clearances per unit of diodrast *T_m* reduces the standard deviations of the former and eliminates most of the difference between the renal plasma flows in men and women.

Correlation of anatomical and physiological studies emphasizes the excretory and secretory functions of the proximal convoluted tubule, and the reabsorptive activity of the loop of Henle and the distal tubule (68). It is difficult to impair reabsorption, even with mercury. The scanty, semisolid urine of chickens is the result of tubular, and not cloacal, reabsorption of water (137).

The conscious dogfish excretes 99 per cent of phenol red via the tubules at plasma levels of 1 mg. per cent or less (251). Hippuran markedly depresses the phenol red excretion, while creatinine has little effect. Creatine excretion like that of creatinine, is 1.5 to 6 times the inulin excretion rate at all plasma levels, but phlorizin does not affect the creatine *T_m* (218).

The tubular excretion of diodrast at both low and high plasma levels is reduced to 60 per cent of normal in dogs moderately phlorizinized (286). The renal plasma flow is unaffected and filtration is not sufficiently diminished to account for the tubular depression.

Excretion of dyes.—Acidification of the perfused frog kidney markedly increases neutral red elimination by diffusion while alkalinization depresses it; neither change affects phenol red excretion (160). Vital staining and dye excretion are unrelated processes. This is also true for fluorescein, which is excreted solely by glomerular filtration in the frog and rat, and acriflavin, which merely stains the tubules (74). The Nussbaum and Ghiron experiments have been confirmed for fluorescein in the frog and rat, suggesting that tubular excretion may be only an emergency function. The excretion of disulfonic acid dyes by the perfused frog kidney depends on the polar structure of the dye; both sulfonate groups must be fixed at one end of the molecule halves (141). This surface action in the excretion of acid dyes is quite different from that of vital staining by basic dyes.

UREA CLEARANCE AND REABSORPTION

The ratio of urea to inulin clearances in the dehydrated chicken with a high ratio of urine to plasma inulin may drop to 0.37, indicating passive back-diffusion of urea with the reabsorbed water

(170). When all filtered water is excreted, all filtered urea is also excreted. Complete reabsorption of urea may occur temporarily with low filtration rates in human diabetic coma (189). On the other hand, in advanced renal disease urea reabsorption diminishes with the falling ratios of urine to plasma inulin or to endogenous creatinine (259). "Reabsorption uremia" is thus rendered unlikely (10).

Massive hemorrhage into the gastrointestinal tract is associated with a low urea clearance (22), a positive nitrogen balance, and increased tissue-protein catabolism during the period of shock, with later rise in blood urea due to formation of urea from the extravasated blood (20). However, in dogs which have been bled or fed blood in single large amounts, azotemia occurs in twelve to twenty-four hours after feeding of blood and is delayed for forty-two to forty-eight hours after the loss of blood alone (158). Little change occurs in the ratio of urine to blood urea. Urea, inulin, and phenol red clearances may be normal or reduced after hematemesis and are unaffected by the feeding of blood in spite of an elevation of the blood urea (260). Renal function, and not the degree of hemorrhage, determines the amount of azotemia (152).

The urea clearance is unaltered by repeated general anesthesia in the dog (200), or by rapid intravenous saline infusion in normal man (93). It is depressed by vomiting (53).

Rats on diets high in protein and low in vitamin A develop a low urea clearance, which rises terminally (133). Carotene increases the clearance. Excess of vitamin A also has this effect in man, especially in the obese subject (135).

The urea clearance of the perfused dog kidney is about half of that in the intact dog with a single explanted kidney (164).

CARBOHYDRATE EXCRETION

The mechanism of glucose excretion in the diabetic dog is the same as in the normal dog, proving that the glycosuria is the result of saturation of the tubular reabsorptive capacity for glucose (107). In human subjects with spontaneous or infusion glycosuria, a similar mechanism obtains except for an elevated "threshold" in advanced diabetes (258), which may prevent glycosuria in diabetic coma with high plasma glucose levels and low glomerular filtration (189). The glucose T_m in normal man is 344 mg. per min. (99).

The ratio of sucrose to inulin clearances in man is reported to be

0.99 and, unlike xylose, is not influenced by glucose reabsorption (257).

Isomannide produces a marked osmotic diuresis in man or dog and is nontoxic (172). (See section on new clearance determinations.)

PROTEINS

The serum albumin and globulin of nephrotic patients are heavier than normal but the corresponding urinary protein fractions are much lighter than the serum proteins in the same individual, this fact indicating the graded permeability of the glomeruli (23). Biological alteration of the nephrotic serum albumin can be shown by the quantitative precipitin test (95). The question arises as to whether these changes are due to excretion of smaller protein molecules, to disturbed regeneration, or to nutritional defect.

Reflex vasoconstriction in pregnant women is followed immediately by proteinuria (50). The "protein filtration rate" varies sharply from hour to hour in "toxemic" pregnant women, but little in nephritic women; this finding suggests vascular "spasms" in the former (46).

Homologous kidney antibodies can be produced in rabbits by the combined injection of kidney and bacterial toxin (236). Similar antibodies are found in the serum of scarlet fever patients, furnishing a possible mechanism for antigen-antibody reaction in the kidney to produce glomerulonephritis. However, homologous nephrotoxic sera have not yet been obtained. An antiserum against renin has been made (151).

Crystalline squash-seed globulin is excreted slightly in the urine of dogs after its injection, without histological renal damage (125). Blood proteins are always excreted simultaneously.

WATER AND SALT EXCRETION AND THE PITUITARY GLAND

Pituitrin in large doses causes temporary arrest of glomerular flow in addition to anuria, the latter outlasting the former considerably (212). Inulin excretion is unchanged during pituitrin oliguria; hence increased tubular reabsorption must occur. Pituitrin increases sucrose diuresis and glomerular filtration in the anesthetized rabbit; maximum tubular reabsorption is reached, as determined by constant depression of the freezing point (211). The concentration of urinary chloride rises and that of sucrose falls. In the conscious dog, one unit of pituitrin increases the maximum

specific gravity of the urine after eighteen hours of dehydration from 1.038 to 1.049, and the urinary concentration of chloride from 2.2 to 7.0 mg. per cc. (210). The "ceiling" of urinary chloride in the rat is 1 per cent; this is obtainable after administration of pituitrin in the rat made diuretic by water or 0.2 per cent saline, in the control rat only after 1 per cent saline given intraperitoneally (244). Water excretion rises as the osmotic effect of chloride increases, but with the use of small doses of pituitrin or partial nephrectomy it can be demonstrated that the chloride-concentrating power is lost before there is any change in the rate of water excretion. Both effects are renal in origin. Pituitrin acts on the kidney to inhibit the reabsorption of chloride and to increase the reabsorption of water.

Salt seems to act differently in diabetes insipidus in the rat than in the cat. In the rat the polyuria is markedly aggravated by sodium chloride or bicarbonate, or Ringer's solution; sodium sulphate or citrate, and potassium or calcium chloride have no excess diuretic effect (264). Nephrectomy prevents polydipsia if the hypophysectomized rat is given water, but not if saline solution is the drink (263). However, salt deprivation alone does not prevent polyuria in the cat with diabetes insipidus due to interruption of the supraopticohypophyseal tracts, while administration of the normal salt content of the diet to an otherwise fasting cat is associated with a marked decrease in urine volume (298). The human subject with diabetes insipidus does not show a diuretic response to a large drink of water nor an increase in urinary chloride concentration after ingesting hypertonic salt solution and elevating the serum chloride level (287). Evidently, the kidney has become less sensitive to changes in the molecular concentration of the plasma. It is unnecessary to assume a disturbance in the tissue-blood exchange of water and chloride in diabetes insipidus.

Pituitrin has no effect on the body water of fresh-water fish although their pituitary glands contain the antidiuretic principle (24). Various reptiles respond with gain in weight, or in body water, but to much lesser degree than the frog (27). This inhibition of loss of body water by pituitrin is more than counteracted by large doses of the drug in birds, mammals, and alligators, if extra water is available (26). The tadpole of the common English toad does not react to pitressin until very late in metamorphosis, when the adult response of retention of water appears, coincident

with suppression of phenol red excretion by the kidney (146). Apparently, the cutaneous and renal sensitivity to pitressin in various species is related to the degree of danger of desiccation.

There is conflicting evidence on the significance of antidiuretic activity of the urine in dehydration and other conditions. Confirming the original report (94) is a study on rats, including data on the lack of influence of sex hormones on the pituitary response to dehydration (184). In addition to direct dehydration, decreases of the serum sodium in cats by adrenalectomy or intraperitoneal glucose injection also leads to an increased antidiuretic effect of the urine (183). However, "strenuous" dehydration is necessary to demonstrate urinary antidiuretic potency in normal cats, although similar technic failed in cats with diabetes insipidus (147). Severe criticism has been directed at the test because of the inconstancy of the finding of antidiuretic activity in the blood or urine of dehydrated rabbits and rats; the lack of difference between hydrated and dehydrated animals after a change in the procedure; the lack of relation between the antidiuretic effect of the cerebrospinal fluid and the state of the pituitary, or the water balance; and the presence of antidiuretic substance in the urine of some hypophysectomized cats and their response to water with diuresis (280). Furthermore, human urine contains no antidiuretic agent; hypophysectomy in the dog does not alter the urinary content of antidiuretic material after dehydration; and the active substance does not increase the urinary chloride concentration nor behave chemically like pitressin (11). An important source of error in all this work is the effect of various common cations (like zinc), anions, and organic substances present in extracts of blood and urine in augmenting the antidiuretic action of pitressin in the rat by delaying absorption from subcutaneous injection sites (197). (Obviously, intravenous injection of suitable extracts of urine is the only reliable technic.)

Edematous, "toxemic" pregnant women excrete an excess of antidiuretic substances in the urine (269), demonstrable even upon intravenous injection into rats (173). The effect is not related to the estrogenic fraction of the urine (269). There is some correlation between the presence of various types of renal and endocrine edema, and antidiuretic activity of the urine (226). Pitressin administration for a week abolishes the latter.

The water load, the blood dilution, and the degree of diuresis in

dogs show a high correlation (4). Water diuresis does not occur in dogs artificially depleted of serum electrolytes, although a large drink of water produces twice as much dilution of the serum proteins as in normal dogs (142). (Plasma volume data are not available.)

That the central nervous system plays a part in water diuresis has been shown in several ways. The relation between the nervous system and water metabolism has recently been reviewed (90, 122). Acetylcholine causes diuresis in the normal thirsting dog and temporary antidiuresis in the atropinized conscious dog; both effects are prevented by hypophysectomy, suggesting that the drug acts centrally to liberate pitressin (217). Destruction of the pituitary stalk abolishes the reflex inhibition of water diuresis upon painful stimulation of the lumbar region or of the pituitary stalk in anesthetized rabbits (126).

The lack of importance of the renal nerves in water diuresis and antidiuresis is evidenced by the identical effect of pituitrin in increasing the creatinine clearance and decreasing the urine volume in both the innervated and denervated dog kidney; and by the equal effect of ether anesthesia in inhibiting the saline diuresis of both kidneys, in spite of the regular vasoconstriction in the innervated kidney under ether (14). Since pitressin does not inhibit saline diuresis, "anesthesia results in inhibition of an inhibition of pituitary inhibition!" Hence, there is no water diuresis from the denervated kidney of an intact dog. No existing theory can account for the known facts of water diuresis. Smith (248) has criticized the theory of "denervation diuresis."

Prior denervation of one kidney prevents the oliguria caused by acutely increased intracranial pressure in dogs and permits diuresis to occur with the rise in blood pressure (230). Denervation causes loss in concentrating power of the kidney without change in the glomerular filtration rate; pitressin restores its ability to form a concentrated urine (128).

ELECTROLYTE CLEARANCES

Contrary to the claim that the discrepancy between the sulphate and inulin clearances in man is due to combination of some sulphate with plasma protein (19), there is cogent evidence that the serum sulphate in dog's blood is freely diffusible *in vitro* and that the low clearance ratio of endogenous sulfate to creatinine,

0.18, represents a true "threshold" (105). The ratio reaches 0.9 at serum sulfate levels above 100 mg. per cent and falls sharply at levels below 30 mg. per cent. The tubules reabsorb 90 per cent of the endogenous sulfate in the dog and much less in man, since his clearance is higher with a normal serum sulfate level only one fourth of the dog's (105). The clearance ratio of sulfate to sucrose in man is unity after intravenous injection of the two solutes; at lower serum sulfate levels tubular reabsorption is evident (219).

The average maximum endogenous potassium clearance in man on a constant intake is 16 cc. per sq. m. at urine flows above the "augmentation limit" of 0.6 cc. per sq. m. (121). Potassium is actively reabsorbed by the tubules at a constant rate. At serum levels up to 100 mg. per cent, potassium acts on the isolated dog kidney to produce diuresis and increased urinary concentration of potassium, sodium, chloride, and urea (148). The excretion rate of potassium, sodium, sulphate, phosphate, and magnesium after intravenous injection in dogs is a linear function of the individual ion concentrations, with tubular reabsorption in every instance (297). The clearances depend on the serum level, the proportion of other ions, and the electroneutrality demands of the urine. (Extrarenal factors are too important in the case of the major electrolytes to justify the expectation of much progress from the standpoint of clearance studies.)

ADRENAL CORTEX AND OTHER ENDOCRINE GLANDS

The typical oliguria, decreased sodium and chloride excretion and increased potassium output in the normal dog can be produced by a single injection of desoxycorticosterone, corticosterone, and dehydrocorticosterone, but not by other crystalline adrenal cortical sterones (271). Progesterone and estrone act similarly, testosterone propionate being much weaker (270). Only desoxycorticosterone and progesterone can maintain life in adrenalectomized dogs. These two sterones also produce diuresis in the normal and especially in the hypophysectomized rat, and increase the resistance to water intoxication (239).

In adrenal insufficiency in the dog the potassium clearance is low, and the creatinine and inulin clearances are equally reduced, but the clearance ratio of urea to creatinine may fall to 0.4 or less (123). Restoration of serum electrolytes rapidly increases the clearances. Sodium depletion in the normal dog acts like adrenalectomy

on the clearances. The renal tubule is the primary seat of the trouble. The earliest change is presumably functional, histochemical changes not occurring in cats, dogs, and rats during the first twenty-four hours of adrenal insufficiency (91). Later there is increased reabsorption of water in the loop of Henle and finally cessation of glomerular filtration. Rats reared on a diet low in sodium behave like adrenalectomized rats in excreting radioactive sodium more rapidly than normal rats (5, 6). Cortin corrects this disturbance.

The antagonism between the adrenal cortex and the posterior pituitary in regard to water and chloride excretion is illustrated by the effect of adrenalectomy in counteracting the acute polyuria of hypophysectomized rats (60). Since pituitrin alone can prevent the hypochloruria in both the hypophysectomized and adrenalectomized rats, the adrenal deficiency seems to be secondary. On the other hand, adrenalectomy does not alter the survival period of cats with diabetes insipidus, nor does pitressin modify the course in adrenalectomized cats or prevent the typical serum electrolyte changes of adrenal insufficiency in cats with both hypophysis and adrenals removed (299). The site of action of pituitrin and the adrenal cortical hormone is in the kidney (244). A state resembling diabetes insipidus has been observed in some normal dogs receiving desoxycorticosterone acetate (224). The disturbance can be corrected by pitressin, this fact suggesting an antagonism of the two hormones in regard to reabsorption of sodium by the tubules. An antidiuretic substance is found in the urine of adrenalectomized cats before there is any change in the serum sodium or potassium; later the amount increases as in other conditions with a low serum sodium (183).

The direct diuretic action of thyroxin on the kidneys has been demonstrated in ingenious cross-transfusion and kidney-transplantation experiments in dogs (39). Thyroxinized kidneys have the same urinary output as kidneys from an untreated dog when transfused by the same recipient, but show a much greater diuresis than normal kidneys if the recipient dog has been hypophysectomized (39). The thyroxinized kidneys have a greater blood flow (37). Blood from thyroxinized dogs decreases the blood flow and urine output of normal kidneys; hence, thyroxin diuresis is not due to a vasodilator in the thyroxinized dog's blood (38). By a similar technique, a purely renal mechanism for the decreased urinary phosphate excretion in parathyroidectomized dogs has been demonstrated (40).

PATHOLOGY

Injection of trypsin into the renal artery produces acute tubular necrosis and renal insufficiency without hypertension (89). The uremia associated with hypochloremic calcium nephrosis in cats with pyloric obstruction is largely extrarenal in origin (100). Marked hydropic degeneration of the convoluted tubules follows repeated daily injection, or a single large injection, of 50 per cent sucrose in dogs (176); similar renal lesions occur in patients and may be a factor in terminal renal insufficiency (7). Glucose and *d*-sorbitol in 50 per cent solutions have no deleterious effect. Severe hydropic swelling of the convoluted tubular epithelium sufficient to cause intrarenal urinary obstruction and fatal uremia follow the ingestion of diethylene glycol in man and several laboratory animals (43). Related glycols, all with an ether linkage, produce similar renal changes and uremia in rabbits and rats (161). Acetylated sulfapyridine precipitates in the urinary tract of the rat, rabbit, monkey, and man in the form of uroliths and can cause anuria and fatal renal insufficiency (8, 262). Ligation of the inferior vena cava between the two renal veins results in oliguria and low function on the congested side and compensatory hypertrophy of the other kidney (292).

A comprehensive study of experimental hyposthenuria in the dog reveals tubular and nontubular types of diminished urinary concentrating power (128). After subtotal nephrectomy the tubules fail to respond to pitressin with increased urinary concentration but react normally to intravenous sulfate injection, increased plasma colloids, or low blood pressure. Hyposthenuria in this case is renal, but nontubular. On the other hand, in uranium poisoning the tubules fail to respond to any measure with increased urinary concentration, because of severe anatomical damage. Low extraction ratios for creatinine and inulin, as well as a low clearance ratio, of creatinine to inulin, suggest back-diffusion of creatinine, and perhaps of inulin. General anemia and renal ischemia (produced by the Goldblatt clamp) have little effect on tubular concentrating function. Ureteral obstruction produces a tubular type of hyposthenuria.

While there is no correlation between the weight of human diseased kidneys and the urea or creatinine clearances, an exponential curve relates these clearances and the number of glomeruli, the latter decreasing more slowly than the former (127). There is a

direct correlation between the number of glomeruli and the maximum specific gravity of the urine until the former has fallen to 35 per cent of normal, when the latter becomes constant at 1.010.

RENAL BLOOD FLOW

Stromuhr studies in dogs and rabbits show that no change in renal blood flow need occur during the reflex oliguria or anuria from mechanical stimulation of the ureter or pelvis (254). The kidney volume shrinks independently of the arterial pressure on the stimulated side, and denervation merely augments the response (12). The kidney has local relative vascular autonomy. Its "reactive hyperemia" is far below that of other organs (232). The renal blood flow is not reduced in experimental nephrotoxic nephritis (231) and the blood pressure is usually not elevated (159). The marked decrease in renal blood flow early in mercury poisoning in the dog can be prevented by immediate decapsulation which relieves the obstruction of intertubular capillaries caused by the rapid swelling of the tubules (177). Small amounts of ephedrine or inhalation of carbon dioxide prevent the late anuria and decrease in renal volume after administration of salyrgan to the chloralosanized dog (62). In hypertensive doses, epinephrine and pitressin inhibit diuresis and decrease renal blood flow; ephedrine inhibits diuresis and slightly increases renal blood flow; and veritol produces diuresis, has no effect on renal blood flow, and can alone relieve anuria due to various types of shock (234). It is evident that nervous inhibition of diuresis can occur in the anesthetized dog without changes in renal blood flow or general arterial pressure. The renal venous pressure and the stromuhr blood flow in the dog change in the same direction after the injection of sympathol or histamine, and remain constant during carotid sinus hypertension and in reflex anuria (255). This parallel behavior under varying conditions of arterial pressure and renal blood flow speaks against any functional significance of the arteriovenous anastomoses in the normal kidney.

Only temporary rises in stromuhr renal blood flow result from rapid intravenous injection of large doses of phenol red and creatinine in the conscious dog (132). Exercise has no effect on renal blood flow or increases it, in spite of the usual oliguria or inhibition of water diuresis (131). Pitressin reduces the renal blood flow in frogs and dogs only if given intravenously (279).

The effect of posture on renal blood flow is noteworthy. The upright position in the anesthetized dog reduces the stromuhr renal blood flow 30 to 40 per cent (187). Studies on five women with nephroptosis during periods of orthostatic hypertension have shown a decrease of 20 to 43 per cent in renal blood flow (as measured by diodrast) in the erect posture, no change in glomerular filtration (as measured by inulin clearance), but an increased "filtration fraction," (188). These results, including the hypertension, are attributed to the action of renin liberated by the postural interference with renal blood flow in the selected cases. On the other hand, change from the recumbent to the semi-upright position by means of a tilting table is associated with reduction in both the renal blood flow and filtration in normal human subjects, indicating that afferent arteriolar constriction has occurred in response to neurogenic stimulation (248).

Slow infusion of renin, the pressor substance in kidney extracts, decreases the renal plasma flow and the clearance ratio of phenol red to inulin and increases the inulin extraction ratio in the conscious dog with an explanted kidney (56). These changes suggest glomerular efferent arteriolar constriction as in human hypertension (247). In the anesthetized dog a single injection of purified renin does not alter the stromuhr renal blood flow (256). The different results in these two reports are not explicable on the basis of the renin preparations or blood pressure variations (57). Prolonged perfusion of the isolated dog kidney under physiological conditions has no effect on renal blood flow, in spite of constriction of the renal artery, until renin production by the kidney has reached an amount capable of causing vasoconstriction within the kidney and a rise in arterial pressure distal to the clamp (164). Here renal ischemia is the result rather than the cause of renin formation. "Angiotonin," the pressor substance resulting from incubation of renin and serum colloids, also constricts the efferent glomerular arterioles but more profoundly than renin, as would be expected from the slow liberation of "angiotonin" from renin (58).

The use of the diodrast clearance in the study of renal blood flow in animals and man is described in inimitable manner by its chief proponent; this development marks an era in renal physiology (247). Diffusion of diodrast between red cells and plasma *in vivo* in the dog introduces a plus error of 13 per cent in the figures for diodrast renal plasma flow as compared with the values ob-

tained from inulin clearance and extraction percentages (285, 289). In man the error is much less (288), although the value of 497 cc. for renal plasma flow is much lower than the earlier figure of 737 cc. (247). The analytical method unfortunately involves the use of a correction factor for incomplete recovery of diodrast (291). In a more representative series of normal humans, the mean diodrast plasma clearance is 688 cc. for men and 600 cc. for women (99). This paper contains a timely critique of the diodrast clearance and related topics.

The explanted kidney in the dog removes only 90 per cent of the diodrast at low plasma diodrast levels (59). Hypophysectomy in dogs reduces the inulin clearance and both the glomerular and tubular fractions of the diodrast clearance to about half of normal, presumably by diminution of renal blood flow (290).

The diodrast renal blood flow in pregnant and post partum women averages 857 cc., indicating the effect of a low hematocrit reading on the blood-flow figure, but little influence of pregnancy on the renal plasma flow (47). In "toxemic" pregnant women, the renal blood flow averages 844 cc., a normal value, but there is twice as much variation from the mean as in the controls (49). Hypertensive nonpregnant women show normal or reduced renal blood flow, with a high "filtration fraction" in the "essential" hypertensive subjects (48). In view of differences in the age distribution of the various groups, the small number in each group, and the calculation of "filtration fraction" from urea clearances, conclusions as to the presence or absence of renal ischemia seem unwarranted. Similar criticism applies to other reports in which the normal controls are much younger than the diseased individuals. On the other hand, pathological changes in the glomerular membrane may increase resistance to filtration enough to counterbalance the augmenting effect of efferent arteriolar constriction on the "filtration fraction" (48).

The influence of the central nervous system upon renal blood flow and kidney function has been relegated somewhat to the background as the result of a study on twenty-one normal subjects under high spinal anesthesia (250). No change in the phenol red, diodrast, and inulin clearances occurs when the subjects are in the prone position. Nor is there "denervation diuresis" in the conscious subject. The normal basal renal vascular tone is not dependent on central nervous sympathetic impulses. Renal vasodilatation, origi-

nating locally, offsets the decrease in general blood pressure during spinal anesthesia. (This paper contains an excellent critical review of the literature.) Psychogenic renal ischemia can be demonstrated under adequate emotional stimulation in man (248).

RENAL METABOLISM AND DIURETIC WORK

The oxygen consumption of the guinea pig kidney *in situ* is twice as high as that of kidney slices and is independent of age and of renal blood flow (15). Slices of ischemic kidney (produced by the Goldblatt artery clamp) have an oxygen consumption 60 per cent of normal (92). On the other hand, no change has been found in the oxygen consumption, respiratory quotient, and aerobic glycolysis in slices of renal cortex from hypertensive dogs or rabbits (185). The oxygen consumption of the perfused kidney of the dog is only slightly reduced after constriction of the renal artery (164). The onset of secretion in the fetal pig's metanephros is associated with cytochrome—cytochrome oxidase activity in the tubular epithelium (82). Adrenalectomy reduces the oxygen consumption of kidney slices (61).

Diethylene glycol nephropathy in the rat does not alter the total oxygen consumption of kidney slices in spite of severe tubular damage, but markedly depresses specific oxidations, carbohydrate synthesis, and ammonia formation, presumably because of alteration in the activating proteins of the oxidizing enzymes (178). Slices and extracts of obstructed kidneys show a reduction in histamine formation and histaminase content (283). (The study of specific metabolic functions of the kidney is in its infancy but promises much for the future.)

Variation in arterial pressure between 70 and 200 mm. Hg has no permanent effect on the arteriovenous oxygen difference in the dog pump-lung-kidney preparation, but oxygen consumption increases with increase of pressure (171). No simple relation exists between urine formation and oxygen consumption. The perfused kidney shows a 38 per cent increase in work and 25 per cent increase in efficiency when the urine volume is doubled by elevating the arterial pressure (69). Urea diuresis is associated with increased renal work and efficiency, but with little change in oxygen consumption. In all types of diuresis, the efficiency increases from the basal of 1 per cent, reaching 5 per cent with urea. "Mechanical" and "tubular" diuresis cannot safely be distinguished. (The dis-

inction between renal excretory work and metabolic or synthetic work deserves careful study in the normal and diseased kidney.) The marked disproportion between the diuresis caused by reduced oncotic pressure and that resulting from increased arterial pressure has stimulated a functional study of "glomerular" and "tubular" (urea) diuresis in the isolated dog kidney and in the anesthetized dog under "isobaric" and "isorrheic" conditions (70). In the isolated kidney, both dilution and pressure diuresis are associated with increased urea and creatinine clearances, in contrast to the constant clearances during urea diuresis. Hence, dilution diuresis is chiefly glomerular. However, in the chloralosed dog there is very little change in creatinine clearance but definite inhibition of tubular reabsorption of water during dilution diuresis. One should no longer distinguish between water diuresis and dilution diuresis. (The striking differences in behavior of the pump-lung-kidney preparation and the intact kidney in the anesthetized dog illustrate some of the pitfalls in renal physiology.)

ISCHEMIC HYPERTENSION AND KIDNEY EXTRACTS

The importance of this phase of renal physiology in the problem of human hypertension (155) justifies discussion in some detail in spite of recent excellent reviews (21, 78, 98, 282). The transplanted ischemic kidney rapidly liberates a pressor substance (29, 79); the kidney *in situ* acts similarly but more slowly (114). It is interesting that no pressor effect is produced by a normal kidney grafted into the neck of a dog nephrectomized two hours previously (79), but definite hypertension occurs if the normal kidney is grafted into a dog nephrectomized two days previously (108). The uremic dog is hyperreactive to normal renal venous blood (106). Experiments on singly and doubly nephrectomized dogs in a variety of ways furnish strong support for the theory that the normal kidney somehow counteracts or neutralizes the pressor substance released by the kidney with constricted arteries (77, 97, 145, 154, 275). This also applies to constriction of the kidney as a whole (111, 203).

Direct demonstration of vasoconstrictor activity in the renal venous plasma of ischemic kidneys has been successful when the test object was a South American toad (79, 144), but has failed in a southern U. S. toad (186). However, perfusion of blood from a heart-lung preparation through a constricted kidney removed from a hypertensive dog can produce a substance that contracts isolated

gut (275) or hind limb vessels (30). Failure to demonstrate a pressor effect in the Locke's solution perfusate of an ischemic kidney (28) or in the systemic blood of hypertensive dogs or patients (86, 138, 154, 222, 278) has been effectively accounted for by the successful elevation of blood pressure in small nephrectomized dogs during continuous, balanced cross-transfusion from large hypertensive dogs (253). A rise in blood pressure has been produced in the dog on intravenous injection of 100 cc. of renal venous blood from a grafted ischemic kidney (29).

Complete obstruction of the renal circulation, or total ischemia, for five or six hours results in the formation of a pressor substance demonstrable on releasing the clamp or ligature or on intravenous injection of the renal venous blood (84, 267). Renal denervation, adrenalectomy, or ablation of the central nervous system does not affect this response (54). Saline perfusates of a totally ischemic kidney have a marked pressor effect in the same animal; no such effect is given by perfusates of the normal kidney or hind limb (223). They apparently contain the same substance as is released into the circulation when blood is allowed to re-enter the ischemic kidney. (It is named "ischemin" but may well be renin.)

The normal kidneys of ordinary mammalian species contain renin (85, 136, 174, 214, 294). This is also true of the dolphin kidney (73). The role of renin in the regulation of normal blood pressure is questionable (277). Its responsibility for the hypertension of renal ischemia is not clearly established by the ordinary assay methods (21, 214, 221). However, the addition of "renin-activator" (162) to renal venous plasma from hypertensive dogs permits the specific detection of renin in that plasma by the vasoconstrictor effect on the perfused ear vessels of the rabbit (201). Also, perfusion from a heart-lung-pump apparatus of the isolated dog kidney reveals a high renin content in the renal venous blood after forty minutes of constriction of the renal artery, but not after four hours of perfusion of the unconstricted kidney (163, 164). On the other hand, the renal venous serum from a heart-lung-kidney preparation, with the renal artery constricted 80 to 90 per cent, is pressor in the dog when injected in only 10 to 20 cc. amounts; it actively constricts the toad's and the dog's hind limb vessels, but does not behave like renin (30, 31). This effect may well be due to "angiotonin" (207). It is possible that different types of perfusion and different degrees of constriction of the renal artery may give

rise to various constrictor substances which may be autolytic derivatives of one another.

In testing the renin theory of renal ischemic hypertension it has been found difficult to maintain an elevation of blood pressure of more than 30 mm. Hg with the slow infusion of renin in normal unanesthetized rabbits for four hours (139). Faster injection leads to a greater, but temporary, rise, and later to a fall in blood pressure to normal in spite of continued injection. Evidence against the renin theory is also adduced from the similarity of the pressor effect of renin in normal and hypertensive rabbits; from the limited sustained rise and the occurrence of tachyphylaxis, or increasing failure to respond when injections of renin are superimposed on a continuous infusion; and from the subsequent return of the blood pressure to the exact preinjection level in both the normal and hypertensive animal (266). These observations have been confirmed in dogs (175, 208) but can be otherwise interpreted. The last point tends to contradict the theory of exhaustion of "renin-activator." Experiments on pithed hypertensive rabbits suggest that renin is not the effective principle in renal hypertension (65). Evidence in favor of renin as the effective agent in renal hypertension is found in a comparative study of the pressor responses to large single doses or continuous injection of renin into conscious trained dogs with normal, ischemic, hydronephrotic, and other types of abnormal kidneys (175). Prolongation of renin effect occurs in those dogs with renal abnormalities which are already unable to counteract their own renin, regardless of the actual blood pressure level.

The mechanism of renin activity has been explained on the basis of activation of purified renin (129)—itself inert in saline solution when perfused through the rabbit's ear—by blood colloids (165, 206). Tachyphylaxis of the rabbit's ear vessels to renin seems to be largely due to exhaustion of "renin-activator" (204). Renin, acting as an enzyme on the substrate of blood colloids, produces the actual pressor and vasoconstrictor substance "angiotonin," which can be crystallized as a picrate and is heat-stable, dialyzable, and alcohol-soluble (207). Further renin action can destroy "angiotonin." Experiments on tachyphylaxis have shown that "angiotonin" also has a specific "activator," that there is "angiotonin-inhibitor" or antipressor activity, that nephrectomy sensitizes the animal to renin and "angiotonin," and that hyper-

tensive animals react like sensitized nephrectomized animals (208). The specificity of renin and its activator has been utilized in quantitating the one with the aid of the other and thus demonstrating an increased renin content of the renal venous blood of hypertensive dogs (201), and an increased "renin-activator" content of plasma from hypertensive dogs and patients (202). "Angiotonin" has been demonstrated in the peripheral and renal plasma of hypertensive dogs and patients by perfusion of the rabbit's ear with small amounts of plasma added to the blood of nephrectomized dogs free from "inhibitor" (205). Finally, renin is liberated by the perfused constricted kidney, presumably as the result of decreased pulse pressure, relative anoxia of the renal tubules, and increased cellular permeability to renin (164). A true vicious cycle results from the renal ischemia in turn produced by renin. (This is a striking series of experiments regardless of the ultimate significance of some of the conclusions drawn from them.)

Confirmation of the view that renin is an enzyme is found in the production of a pressor substance, "hypertensine" (resembling "angiotonin" in most of its properties), by the interaction of renin and normal serum (31). A similar substance can be extracted with acetone from renal venous serum of acutely ischemic kidneys grafted into the neck of a dog or perfused by a heart-lung preparation (32). However, the purest renin so far prepared has no ordinary enzyme activity (300).

The problem of tachyphylaxis to renin is still puzzling. Purification of renin does not eliminate tachyphylaxis (129, 136, 174, 175, 190, 265). The chief obvious factor is the time interval between injections (190). It is very significant that "renin-activator" does not alter the refractoriness of the dog or cat, nor does it relieve tachyphylaxis in the perfused rabbit's ear, if a tachyphylactic dog's blood is injected with the activator and renin (204). Elimination of central nervous influences, transfusion of blood in large amounts, and the use of various drugs have no influence upon tachyphylaxis. As a result of this and other types of evidence suggesting that the kidneys of normal and renin-refractory animals give off substances with inhibitory or antipressor activity, new depressor extracts have been obtained from kidney and muscle, effective orally and parenterally but only in animals with renal hypertension (116, 124, 209). Subcutaneous implants of kidney can apparently release antipressor substance (228). The importance of

this work in relation to human hypertension is obvious but at the present writing the clinical experiments do not permit definite conclusions (117).

The site of action of renin is on the peripheral vessels (1, 85, 136, 193, 204). Renin does not act on the isolated heart (136, 140). It is ineffective on intracisternal injection (204). Renin is not a sympathomimetic drug (153). The pulmonary arterial pressure is normal in renal hypertension (156). Renin produces diuresis and increased excretion of sodium and chloride without change in the creatinine clearance in the unanesthetized rabbit (216). That the vasomotor center may be sensitized in renal hypertension by something else than renin is shown by the exaggerated response of the pithed hypertensive rabbit to epinephrine but not to renin (65). Hypertensive animals are hypersensitive to tyramine and pitressin (35, 198, 275). Adrenalectomy decreases response to renin (87, 204, 293) but does not prevent the hypertension of renal ischemia (229).

Renal extracts may contain tyramine (294), but none is demonstrable in the blood of hypertensive dogs (275). Tyrosinase lowers the diastolic pressure in hypertensive rats (235). Anaerobic kidney extracts produce oxytyramine, a pressor substance (143). Anaerobic autolysis of dog kidney cortex yields a powerful pressor filtrate which has a more prolonged effect on hypertensive than on normal dogs, and less on nephrectomized dogs; aerobic autolysis produces a strongly depressor material (276). (The relation between these extracts and the mechanism of renal hypertension is intriguing but still somewhat remote.)

The widespread arteriolar necrosis and hemorrhages observed in "malignant" human and experimental renal hypertension have been correlated with renal insufficiency (97, 98, 296), except in the rat (295). Uremia without hypertension is not associated with these lesions. A comparative study of the effects of various renal extracts when injected daily into dogs without kidneys shows that widespread lesions can be produced similar to those seen in hypertensive dogs with necrotic ischemic kidneys, that hemorrhages and edema result from the depressor and vasodilator fraction of the crude kidney extract, while the necrotic lesions are produced by the pressor, or renin, fraction (300). (This is a very stimulating line of approach toward the chemical renal etiology of the manifold aspects of cardio-renal-vascular disease.)

Purified renin is apparently a protein, coming down with the pseudoglobulin fraction (53, 118, 129, 136, 175, 214, 265, 300). The purest preparation gives a rise of 30 mm. Hg in the blood pressure of the dog on the injection of only 0.001 mg. of nitrogen per kg., and even this material contains 75 per cent of inert globulin (300). It is free of ordinary enzyme activity.

RENAL HYPERTROPHY AND NECROSIS

The relative rate of kidney growth in the rat, normally constant, is increased by high protein diet and thyroxin, and is decreased by low protein diet, starvation, pregnancy, and thyroidectomy (281). Compensatory hypertrophy after unilateral nephrectomy in the rat is complete in forty days, reaches 70 per cent, and is independent of age (3). Complete hypophysectomy prevents this compensatory hypertrophy in young dogs (301), frogs (101), and rats (191), while thyroidectomy and low caloric intake do not depress it (191). Hypophyseal implants stimulate renal hypertrophy in frogs (101).

Castration in the male rat causes a decrease in the weight of the kidney and in the size of the proximal convoluted tubule; androgens restore the kidney and induce excess hypertrophy (169). Androgens produce marked hypertrophy in the female kidney, but not in that of the male; estrogens have only a toxic effect on the tubules. The androgen effect predominates over the estrogen action in combined injections. Chemical and histological confirmation of these effects on the renal cortex have been reported (213). Female mice are more responsive than rats to androgens (237). Pretreatment with androgens given to produce hypertrophy protects the mouse kidney against mercury poisoning (238).

The hemorrhagic necrosis of the kidneys in rats on a low choline diet (112) is dependent on the ratio of methionine to cystine (113). Similar lesions develop with diets low in vitamin B₆ and deficient in choline (120).

NEW CLEARANCE DETERMINATIONS

Comparison of the inulin, creatinine, and urate clearances in gouty patients shows that the normal reabsorption of 90 per cent of urates by the tubules is not diminished until the inulin clearance is less than 50 per cent (55). Cinchophen and salyrgan increase the

urate clearance markedly by depressing tubular reabsorption; colchicine has no effect. The renal changes are probably the result and not the cause of the metabolic disturbance. A systematic review on uric acid metabolism and excretion has been written by Brøchner-Mortensen (33).

The clearance of hemoglobin in the dog averages 2 per cent of the creatinine clearance at plasma hemoglobin levels of 200 to 1300 mg. per cent, above which there is direct proportionality between excretion and plasma concentration (195). There is no tubular excretion; the T_m (reabsorption) is 2.0 mg. per min. with a glomerular filtration of 1.23 cc. per min., or 3 per cent of the creatinine filtration rate. Presumably 3 per cent of the glomerular pores are large enough normally to let hemoglobin filter through.

The vitamic-C excretion in the dog, as in man (225), is determined by glomerular filtration and active tubular reabsorption, the latter reaching a maximum, T_m , of only 0.52 mg. per 100 cc. filtrate in contrast to 1.7 mg. for man (243). The tubular mechanisms for reabsorption of glucose and vitamin C are different in the dog, as in man. Reabsorption of ascorbic acid is never complete in man, even at very low plasma levels (88).

The clearances of sorbitol, mannitol, dulcitol, and sorbitan are identical with the inulin and creatinine clearances in the dog and with the inulin clearance in man, thus furnishing another measure of glomerular filtration (252). Isomannide and sorbide are reabsorbed to the extent of 50 per cent by a tubular mechanism different from that of glucose.

MISCELLANEOUS

It is regrettable that limitation of space prevents detailed discussion of the following papers: the influence of denervation of the kidney on salt and water excretion in the conscious dog (109); effects of high and low protein diets on the course of nephrotoxic nephritis (245, 246) and of subtotal nephrectomy in rats (45, 76); toxicity of potassium in rats with renal insufficiency (2, 18); action of diuretics on the normal and poisoned frog kidney (149); effect of blood and gum acacia infusion (166) and of acid and alkali administration (167) on the creatinine clearance in normal and nephropathic rabbits; increased glucose utilization after nephrectomy in hepatectomized rabbits (17); muscle water in hydronephrotic dogs (71); blood pressure in hydronephrotic dogs (72, 192); uri-

nary excretion changes in the "non-adapted" rat (36); organ weights and histology in hypertensive rats (64); effect of renal function on the vitamin-C clearance in man (240); effect of denervation and adrenalectomy on anoxic oliguria (272); nonexcretion of the renal pressor substance (227); effect of urinary solutes on the specific gravity (220); hypnotics and renal function in rabbits (168); parallelism of urinary ammonia and amino acid coefficients (83); changes in plasma volume and proteins after diuretics (42); hypertension and increased blood volume in triply nephrectomized parabiotic rats (150); x-ray studies on the mobility of kidneys in young adults (196); pituitrin inhibition of water loss in rats (25); effects of accessory blood supply on renal function and hypertension in dogs (44, 63, 96, 181, 182); excretion of indican as a renal function test (233); constancy of urinary pH in man on various diets (34); absence of pressor effect from renal extracts of hypertensive, subtotally nephrectomized rats (16); effect of renal denervation on neurogenic hypertension in dogs (115); tubular reabsorption of sodium sulfapyridine and sulfanilamide, with simple glomerular filtration of glucose sulfapyridine (268).

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METABOLIC FUNCTIONS OF THE ENDOCRINE GLANDS¹

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This attempt at a brief review of the contributions of the past year to our knowledge of the metabolic functions of the endocrine glands must of necessity be selective rather than all-inclusive. This is not only because of the number of papers which have appeared, but also because many of the findings cannot yet be related to the pre-existing knowledge. For these reasons it has seemed desirable to limit the present discussion to the most pertinent literature concerning the influence of the pancreas, the adrenal cortex, the thyroid and the anterior hypophysis on the metabolism of carbohydrates, fats, and proteins respectively. 222

Since it is possible to make only the briefest mention of the related work which appeared before the period under consideration, the reader is referred to certain review articles published during this time. Van Dyke's second volume on the pituitary gland (144) covers the international literature on the subject from 1935 to 1938. Collip (28) has discussed the significance of the various active fractions of anterior pituitary extracts. The influence of the pituitary gland on carbohydrate metabolism has been reviewed by Young (154), and of the adrenal cortex by Long and associates (83). Raab (110) has considered the relation of the hormones to fat metabolism. ← note

It is understood that the interpretation of new observations will often depend upon one's previous conception of the fundamentals of the subject. The writer has recently summarized some of his views on carbohydrate metabolism (126, 127).

THE PANCREAS

A more complete understanding of the influence of other hormones on carbohydrate metabolism still awaits final agreement as

¹ The help of Dr. R. Levine in the collection of the material for this review is gratefully acknowledged.

to the functions of insulin, and as to the significance of the diabetic state. Towards this end, there has been much work and an increasing use of the methods of experimentation *in vitro* elaborated by Warburg, Krebs, and others. The action of insulin which has been most generally agreed upon is its effect in increasing the storage of muscle glycogen. Gemmill (47) has finally demonstrated this action of insulin *in vitro* on rat diaphragm in the presence of glucose. We have confirmed this work in our laboratory (unpublished) with the added observation that the magnitude of the catalytic effect is inversely proportional to the concentration of glucose present. As the latter approaches 400 mg. per cent, little if any accelerating effect of insulin is noted. This relationship between insulin action and the sugar concentration should be considered along with that previously demonstrated for carbohydrate utilization (128). Here too, the presence or absence of insulin made little difference, once certain high levels of blood sugar were reached. In view of the increasing knowledge of the chemical mechanisms of glycogenesis (31), Gemmill's observation should open the way to the elucidation of insulin action in this regard.

The influence of insulin on glycogenesis in the liver has been confused by the fact that, while it increases hepatic glycogen stores in the diabetic organism, its injection into normal animals (without coincident sugar administration) leads to decreased liver glycogen. Previous work by Bridge (18) in intact normal animals and by Lundsgaard (87) in perfused livers had failed to demonstrate any action of insulin on hepatic glycogen deposition. However, Seckel (118) had reported an inhibition of glycogenolysis in liver slices by insulin, while Soskin *et al.* had demonstrated the influence of insulin on the hepatic mechanism for the regulation of the blood sugar level. The latter authors have recently reported (133, 141) a demonstration *in vitro* of the primitive basis for this homeostatic mechanism. The appearance of free sugar in glycogenolyzing liver brei was inhibited by the addition of glucose; the extent of inhibition was determined by the amount of added glucose; and the inhibition resulting from a given concentration of glucose was potentiated by the administration of insulin to the animal before the liver was removed. Insulin added to the liver brei *in vitro* was without effect. They have since been able to demonstrate (in unpublished work) a similar effect on liver slices respiring *in vitro*, when the disappearance of glycogen rather than the appearance of

free sugar was determined, and when insulin was added directly to the Warburg vessels.

With regard to the utilization of carbohydrate, Soskin and co-workers (134) have repeated some of their chemical balance experiments in eviscerated dogs, in order to evaluate certain possible objections to their methods of estimating and calculating the amounts of carbohydrate used. The close correspondence of utilization rates, obtained by different methods and by different calculations, strongly supported the validity of the chemical balance method as a means of determining carbohydrate utilization in liverless animals. Using this method, Dye & Chidsey (38) have confirmed the work of Soskin *et al.* (128) which demonstrated that the peripheral tissues of the diabetic dog utilize sugar, and that in both normal and depancreatized dogs the rate of carbohydrate utilization depends upon the height of the blood sugar level. More recently, Greeley & Drury (51), working with eviscerated and hepatectomized rabbits, have also concluded that diabetic tissues can and do utilize glucose and that, under basal conditions, the glucose utilization rate of diabetic tissues is the same as that of normal. Their results probably warrant the first conclusion, but are not sufficiently quantitative to justify the second, for their data were derived merely by subtracting the amount of sugar excreted by the kidneys from the amount of sugar which had to be administered to maintain the blood sugar level of their liverless animals. No carbohydrate balance sheet was drawn to include changes in muscle glycogen and in the lactic acid content of blood and muscle. These were omitted because of the entirely unfounded belief that the muscle glycogen and its breakdown products, including lactic acid, cannot be utilized by the muscle in the absence of the liver. It is therefore not surprising that they found it difficult to explain why one group of animals (four fed, one fasted) with presumably good glycogen stores maintained constant rates of sugar withdrawal from the blood for at least six hours, while another group (one fed, four fasted) with poorer stores began to draw more heavily on the blood sugar after three hours. It is also clear why they found that the amount of glucose required to maintain the blood levels of their animals accounted for only one sixth to one third of the energy requirements of the animals as judged by oxygen consumption. Best, Dale, Hoet & Marks showed, many years ago, that the sugar which disappeared from the blood

of eviscerated spinal cats was equal to the sum of the glycogen deposited in the muscles and the glucose equivalent of the oxygen consumed.

The chemical balance work referred to above had shown that the presence of insulin in normal animals enabled them to utilize amounts of carbohydrate at low blood sugar levels, for the use of which diabetic animals required hyperglycemic levels. Soskin *et al.* (129) studied the effect of large doses of additional insulin on the sugar utilization of eviscerated normal animals. They found that the administered insulin did not increase the rate of sugar utilization at any blood sugar level, although it did increase the storage of muscle glycogen. Thus the amount of insulin available in normal animals is apparently sufficient for the maximal rate of glucose catabolism, but not sufficient for maximal glycogen synthesis. From this, and from the previous work on depancreatized animals, it was concluded that insulin is not primarily concerned with the oxidative breakdown of carbohydrate. It probably facilitates the formation from glucose of an intermediate substance (possibly a phosphorylated hexose), which precedes and is necessary for both catabolism and synthesis.

The conclusions drawn from recent experiments *in vitro* partly agree and partly differ as to the nonessential nature of insulin in carbohydrate oxidation. However, the acceptability of the evidence from experiments *in vitro* is not yet clear. Continuing their studies on the metabolism of diabetic tissues, Shorr *et al.* (121, 123) have reported that cardiac muscle from depancreatized dogs does not oxidize glucose when first placed in the Warburg apparatus, but gradually assumes this ability when kept in the vessels for from five to ten hours. This conclusion was based on the progressive rise in the respiratory quotient (accompanied by a fall in creatine phosphate) which they observed over these long periods of time. They have concluded that insulin and other hormones are not essential to the enzymatic processes by which carbohydrate oxidation is brought about, but that they act as accelerators or depressors on the rates of these processes. The delayed appearance of glucose oxidation in the excised diabetic cardiac muscle *in vitro* was explained by postulating a gradual degradation of the hormones carried over with the tissue, and an escape of the tissue from the inhibitory influences (possibly anterior pituitary and adrenal cortical) originally present. Attractive as this explanation may seem,

it cannot be reconciled with the fact that in the depancreatized dog the blood sugar begins to fall as soon as the liver is removed, so that profound hypoglycemia occurs within two hours. Thus the "escape," if such it be, would have to occur much faster in the whole animal than in isolated tissue slices, a supposition which is opposed by general experience. The fundamental objection to the work of Shorr *et al.* is their use of the respiratory quotient as an index of carbohydrate oxidation, without adequate supporting evidence as to the enzyme systems which are involved, the substrates which disappeared, or the intermediates which accumulated. This is discussed further in Soskin's review (127). It is more than possible that their low initial respiratory quotients did not signify a lack of carbohydrate oxidation, and that the subsequent rise meant nothing more than the cessation of some enzymatic reactions with low respiratory quotients, which might be expected to occur during the disorganization of tissues kept under artificial conditions for such unusually long periods of time.

In 1938 Krebs & Eggleston (76) had observed that the respiration of pigeon breast muscle *in vitro*, when supplemented with glucose and dicarboxylic acids, was maintained for longer periods of time when insulin was added. They concluded that insulin was a limiting factor in the oxidation of glucose by way of the citric acid cycle. From this they arrived at the generalization that insulin was essential for the oxidation of carbohydrate, and that the non-utilization theory of diabetes was therefore supported. Attempts to confirm and extend their original observations on pigeon breast muscle, have yielded results which are qualitatively similar (122, 137, 139) though not always as significant quantitatively (122, 137). However, it has not been possible to repeat their observations on the muscles of normal chickens, dogs, rats (122), or cats (122, 137). Stadie *et al.* (137) also failed to demonstrate the effect on the muscles of depancreatized cats and pigeons. On the other hand, Stare & Baumann (139) reported that insulin (but not "inactivated" insulin) did maintain the oxygen consumption of depancreatized pigeon muscle, and that the effect was inhibited by malonate. But this held true only for pigeons depancreatized for periods of four days to three weeks. No effect was obtained on the muscles of pigeons depancreatized for a longer period! It should also be pointed out that, of all creatures, the pigeon is one of the most resistant to the known physiologic actions of insulin, and that

the removal of the pancreas from this bird, as from the duck (100), results in very little change in the blood sugar level or in the urinary sugar excretion. For the time being, it therefore seems proper to regard the observation of Krebs & Eggleston as an isolated phenomenon with very limited general application, occurring in an organism in which insulin does not seem to play its usual important role.

The previously demonstrated influence of insulin on protein metabolism has been confirmed in a variety of ways. Bach & Holmes (7) had shown that the gluconeogenesis observed in excised liver slices *in vitro* was reduced by insulin, and that this diminution was accompanied by a depression of urea formation. Stadie *et al.* (136) have confirmed the fact that insulin inhibits the oxidative deamination of amino acids, although they found that the inhibition was not exerted on the *l*-amino acids, and occurred consistently with the *d*-amino acids. Their results differed from those of Bach & Holmes in that they found no influence of insulin on carbohydrate synthesis. Similarly, they observed an increased formation of urea in slices from the livers of diabetic cats, but found no increased gluconeogenesis. In fact, they reported that the diabetic liver slices produced less carbohydrate than normal slices, either spontaneously or in the presence of added *dl*-alanine. It is difficult to account for these results, for the increased gluconeogenesis of the diabetic liver (and particularly from protein) has been well established *in vivo*.

In 1938 Mirsky (95), by determining the rate of nonprotein nitrogen accumulation in the blood of nephrectomized animals, demonstrated that insulin decreased the rate of protein catabolism. From the fact that insulin also lowered the nonprotein nitrogen and the amino acids of the blood in the absence of the liver, he concluded that insulin exerts its protein-sparing effect in two ways. It increases protein synthesis from amino acids in the muscles, as well as inhibiting deamination in the liver. Farr & Alpert (42) and Crismon *et al.* (32) have again shown that insulin lowers the blood amino acids. These authors have also reported that epinephrine produces the same effect. It seems likely that this is not a direct action of epinephrine, but is due to a reflex secretion of insulin. For, as we shall see in our discussion of the hypophysis, anterior pituitary extracts have a nitrogen-sparing action in the intact animal (45) which persists in the absence of the adrenals (57), but not when the pancreas is removed (46, 96).

The protein-sparing action of insulin has been shown in still another way by MacKay *et al.* (88). In rats maintained on a diet containing 20 per cent casein and 41 per cent starch, the administration of insulin caused the nitrogen balance to become more positive, whether the food intake was fixed in amount or was given *ad lib.*

Within recent years, beginning with the work of Chaikoff & Soskin (25) and culminating in the work of Mirsky *et al.* (97, 98), there has accumulated much evidence that the rate of utilization of the ketone bodies by the peripheral tissues bears no relationship to the presence or absence of insulin or to the rate at which carbohydrates are being utilized. These observations have now been further substantiated by Dye & Chidsey (38) in dogs, by Barnes *et al.* (10) in dogs and rabbits, and by Stadie *et al.* (138) *in vitro*, on minced muscle taken from normal and diabetic cats. Indirect evidence in support of the same conclusions has also been reported by Barker (9), although this author suggests a less obvious and more labored explanation. Using depancreatized dogs, he increased the total metabolism and presumably the rate of turnover of fatty acids by means of exercise, thyroid, and dinitrophenol and found, contrary to his expectations, that there was no increase in ketonuria.

The early work of Embden had indicated that the liver was the chief if not the only source of ketone bodies, and some of the work mentioned above had confirmed and extended this observation to show that when glucose or insulin exerted an antiketogenic action, it did so by inhibiting the formation of ketones by the liver. Baer (8) has found that the ketonuria resulting from the intravenous injection of butyric, isovaleric, or acetic acids is not influenced by either glucose or insulin. It therefore appears that the antiketogenic action of the latter substances must be exerted higher up in the chain of reactions leading from fatty acids to the ketone bodies. Stadie *et al.* (138) have studied ketogenesis and antiketogenesis *in vitro* on liver slices from normal and depancreatized cats. They found that the diabetic liver slices produced ketones at a rate four to five times that of the normal, and that the diabetic slices yielded the correspondingly low respiratory quotient of 0.32. Insulin, in the presence of fructose, inhibited the ketogenesis as much as 42 per cent. From the ratio of ketones produced to oxygen consumed by the diabetic liver slices, Stadie *et al.* calculated that four molecules

of ketone were formed from each sixteen-carbon-atom fatty acid, with too little oxygen left over to allow for any carbohydrate formation from the fatty acids. This may have been so under the conditions of their experiments, but does not warrant the conclusion that gluconeogenesis from fatty acids may not occur under other conditions. For it should be pointed out that, if their conditions were indeed such that the entire molecules of the fatty acids were converted to ketones, no parts would have been left for conversion to carbohydrate. Under these circumstances the amount of excess oxygen consumption should have made little difference to their conclusions, unless it were assumed that gluconeogenesis from fatty acids went by an entirely different pathway than ketone formation. This is not necessarily so in view of Weil-Malherbe's demonstration (147) of the formation *in vitro* of sugar from acetoacetic acid by kidney slices. It should also be pointed out that Blixenkrone-Møller (16), in studies of perfused diabetic livers in which gluconeogenesis from fatty acids was found to occur, calculated that varying numbers of molecules of ketone (from one to four) could be derived from a molecule of fatty acid at different times.

An interesting bypath of insulin function, aside from its effects on the metabolism of the common foodstuffs, has been its supposed influence on the utilization of alcohol. Lundsgaard (87) had failed to find any utilization of alcohol by the perfused hind limb. Mirsky & Nelson (99) have confirmed the fact that the peripheral tissues are not involved in alcohol utilization by following the rate of removal of alcohol after its intravenous injection into eviscerated dogs. They also found that even large amounts of glucose and of insulin did not affect the rate of alcohol disappearance in such preparations. In intact dogs they showed that fasting and chloroform anesthesia depressed the rate of alcohol disposal. Chronically depancreatized animals had a slower rate of utilization than freshly depancreatized dogs which showed the normal rate. The authors concluded that insulin affects the utilization of alcohol indirectly, and only to the extent that it maintains a normal state of the liver by limiting the degree of fatty infiltration.

The inclusion of lipocac here, as an internal secretion of the pancreas, depends upon the work of Dragstedt and co-workers (35, 36, 37). Their contentions, supported by a great deal of careful evidence, are based upon their findings: (a) that ligation of the external pancreatic ducts does not result in the characteristic fall

in serum lipoids and the rise in liver fat which occurs in depancreatized dogs not receiving raw pancreas, lecithin, or choline in the diet; (b) that administration of large quantities of the external secretion of the pancreas, collected from pancreatic fistulae, does not alleviate the above changes in depancreatized animals; and (c) that their simple alcoholic extract of pancreas, named lipocaic, is very potent in relieving the fatty changes, and does not contain sufficient choline to account for the results. The first two observations have now been challenged, both by Chaikoff and associates (40, 101) and by Rubin & Ralli (113), whose results appear to be directly contrary to those of Dragstedt. If the work of these dissident authors is correct, lipocaic would have to be regarded as an external pancreatic secretion. There seems to be no basis at the present time for deciding between or accounting for the conflicting versions as to the facts of the case.

Another disputed question, whether the activity of extracts of lipocaic might be accounted for by contained choline plus the choline and protein contents of the diet ingested by the test animals, has now been settled. Dragstedt and co-workers (72) have demonstrated a difference between the actions of lipocaic and of choline in regard to the alleviation of the fatty infiltration of the liver which followed the administration of ketogenic extracts of the anterior pituitary gland to guinea pigs. Lipocaic reduced the fat content of such livers while choline did not. McHenry & Gavin (93) have shown that fatty livers can also be obtained in rats by the administration of an alcohol-soluble fraction of liver. Here again lipocaic effectively reduced the fat content while choline did not. In view of the high initial cholesterol content of the livers in their rats, and the effect of lipocaic in reducing it, McHenry & Gavin have suggested that lipocaic may be particularly concerned with cholesterol transport, while choline activity is directed towards neutral fat. This offers an interesting and helpful working hypothesis in the attempt to establish the true relationship between these two active substances, with their puzzling similarities and differences.

In recent years Macallum, Laughton and co-workers have been the chief proponents for the existence of a duodenal hormone, acting synergistically with insulin and antagonistically against anterior pituitary extracts, in the regulation of carbohydrate metabolism. Their basic conceptions have depended chiefly on their

observation of reductions in the blood sugar level following stimulation of the duodenal mucosa with dilute hydrochloric acid, and following the injection or oral administration of extracts of the duodenal mucosa. Loew, Gray & Ivy have failed to confirm this work. They did not find any reduction in the fasting blood sugar level of dogs after stimulation of the duodenal mucosa with dilute acid (80). Nor did such treatment enhance the utilization of intravenously administered glucose, or reduce the hyperglycemia produced by the absorption of glucose, the injection of epinephrine or the removal of the pancreas (81). Finally, they were unable to affect the blood sugar level of dogs with various extracts of the duodenal mucosa which were made according to all the methods previously advocated (82). This work casts serious doubts upon the existence of a duodenal hormone which is liberated after stimulation of the duodenal mucosa and which participates in the regulation of the blood sugar.

THE ADRENAL CORTEX

Previous work on the adrenal cortical hormone has made it sufficiently evident that, whatever its natural structure, extracts of the gland may not be regarded as containing a single active substance. Hartman *et al.* (61) have reported that they find two factors in adrenal cortical extracts which potentiate each other but which have largely separate actions. One maintains the sodium levels of the tissues, but is relatively ineffective in maintaining appetite and normal behavior and in preserving life in adrenalectomized cats. The other factor ("cortin") is very potent in preserving life, appetite, weight, and normal behavior even while the serum sodium remains low. In the light of other work, however, the views of Hartman *et al.* would seem to represent an oversimplification of the problem, and to minimize the importance of the sodium and potassium balance for the well-being of the living organism.

The isolation and identification of a number of steroids (39, 73, 92) from the adrenal cortex, and the study of their physiological properties and those of the amorphous fractions, have revealed that the various compounds or fractions have certain activities in common. However, a particular compound or fraction may exhibit one activity to the highest degree, and be relatively impotent in other respects. In the absence of more precise knowledge of that vital function, the failure of which is the most urgent cause of death

in untreated adrenalectomized animals, it is convenient to compare the various cortical steroids and fractions in respect to their following effects on such animals: (a) the maintenance of life; (b) the restoration of normal carbohydrate levels in all tissues; and (c) the restoration of normal sodium and potassium balance and excretion. To these effects may be added the restoration of the ability of the muscles to continue to perform work in response to prolonged stimulation, according to the test developed by Ingle. But since the activities of substances in this respect run parallel with their carbohydrate effects, these two actions may be considered together.

Kendall's amorphous fraction (cortin) and his desoxy B compound seem to be the most potent for maintaining life (73, 74). The carbohydrate levels are best restored by corticosterone and its derivatives with an oxygen on C₁₁ (49, 73). In this respect, cortin has some effect, but desoxycorticosterone has very little (83). The relative potencies of the substances acting on carbohydrate levels maintain a similar relationship when these materials are tested on muscular work performance (67, 68, 69). Some of the earlier work with synthetic desoxycorticosterone acetate, while showing its powerful influence on the sodium and potassium balance, had revealed no action on carbohydrate metabolism (17, 43). This is apparently a matter of dosage, for Harrison & Harrison (59) have reported that 1.25 mg. daily of the substance would maintain life and a normal mineral balance in adrenalectomized rats, but that it required 2.5 mg. daily to maintain a normal blood sugar level. Similar evidence is available in the work of Britton & Corey (19), Ingle (68), and Long, Katzin & Fry (83), although these authors differ from Harrison & Harrison and from each other as to the comparative potency of desoxycorticosterone on carbohydrate metabolism.

In spite of the qualitative difference in the prepotent activity of the various substances which may be separated from adrenal cortical extracts, it is impossible to discuss the materials concerned with the metabolism of the foodstuffs without also considering those which primarily affect the mineral balance. This is because the absence of the latter in adrenalectomized animals disturbs the normal environment of all cells and thus produces certain secondary disturbances in metabolism. The secondary effects are most readily distinguished from the primary metabolic effects of adrenalectomy by a consideration of those disturbances which are al-

leviated by combating the mineral imbalance with a high sodium and low potassium intake. Thus it has been shown that the diminished rate of glucose absorption by the intestines after adrenalectomy is completely restored to normal by the administration of sodium chloride in the drinking water (3). The same holds true for fat absorption (27). Salt-treated adrenalectomized rats can deposit glycogen from glucose as well as normal rats (4) and may gain weight in normal fashion (65). Britton & Kline (20) have reported that adrenalectomized cats do not deposit glycogen even at high blood glucose levels and after insulin administration, unless cortin is given. In view of the foregoing, however, it seems likely that their results might have been different in adrenalectomized animals receiving a diet high in sodium and low in potassium.

The observations of the normal absorption of carbohydrate and fat in salt-treated adrenalectomized animals (3, 27) are directly opposed to the theories of Verzář. This author, starting with his observation that the intestinal absorption of the foodstuffs was diminished after adrenalectomy, had related this defect to a disturbance of the phosphorylating mechanisms, and had assembled rather impressive evidence that the adrenal cortex was primarily concerned with phosphate transfer. Recent attempts to confirm his findings and conclusions have been almost uniformly unsuccessful (22, 26, 107, 114).

What, then, are the primary functions of the adrenal cortex in respect to the metabolism of the foodstuffs? The answer appears in those metabolic disturbances in the adrenalectomized animal which persist despite the maintenance of a normal sodium and potassium balance. Although well-fed adrenalectomized rats and mice maintained on a high sodium intake exhibit normal carbohydrate stores, fasting adrenalectomized animals suffer a sharp decline in the carbohydrate levels of all tissues despite salt treatment (43, 83). Treatment with corticosterone or cortin (19, 60, 83, 116) restores the normal blood sugar level and, in large doses, may cause hyperglycemia. Such treatment also increases the liver glycogen in normal as well as in adrenalectomized animals. The muscle glycogen is not so readily affected either by adrenalectomy or by the administration of cortical extracts. Recent work has also confirmed the previous reports that the lack of adrenal cortical hormone diminishes the hyperglycemia and glycosuria of diabetes (17, 83), and that the administration of active cortical hormones

restores the severity of the diabetic syndrome (17, 68, 83). Similarly, Wells (149) has reported that the injection of phlorizin into salt-treated adrenalectomized rats causes them to excrete much smaller amounts of glucose than similarly injected normal rats. Corticosterone and 17-hydroxy-11-dehydrocorticosterone (compound E) increase the glucose excretion of the phlorizinized adrenalectomized animals to that of phlorizin-treated normal rats. The amorphous fraction ("cortin") and desoxycorticosterone have relatively lesser effects. It may therefore be concluded that the primary metabolic functions of the adrenal cortex are concerned with hepatic gluconeogenesis from noncarbohydrate precursors. The observation of Corey & Britton (30), that cortin retards the fall of glycogen in perfused livers, also suggests an antiglycogenolytic activity of the adrenal cortex. This may explain the more marked effects of cortical extracts on liver glycogen as compared to muscle glycogen. It also helps to distinguish the action of these extracts from those of the anterior hypophysis (116).

From their observations on the effect of cortin on the respiratory quotient of glucose-fed adrenalectomized animals, Long, Russell and co-workers (83, 116, 117) have supposed that the adrenal cortical hormone may depress carbohydrate oxidation. This conclusion is subject to the usual objections which apply to such use of the respiratory quotient (127). Moreover, Selye & Dosne (120) have shown that while cortin will inhibit the fall in blood sugar of partially hepatectomized rats, it fails to have any effect in completely liverless animals. It is apparent, therefore, that cortin does not inhibit the uptake of sugar by the peripheral tissue, but probably stimulates gluconeogenesis in the liver. It is suggested that its tendency to counteract insulin hypoglycemia (119, 120) is exerted in a similar manner.

The probability that the low carbohydrate levels in the fasting adrenalectomized animal are not due to an increased carbohydrate oxidation is enhanced by the demonstration of an impaired work performance of the muscles. Ingle (65) has shown that the work performance is markedly diminished in adrenalectomized animals, even when they are maintained in apparently good condition by a diet high in sodium and low in potassium. This effect is due wholly to the loss of the adrenal cortex, for removal of the adrenal medulla has no influence (56). The comparative effects of the closely related cortical steroids on this phenomenon have also been studied (69). It

is significant that the work capacity of the gastrocnemius muscle of the rat is decreased even after a unilateral adrenalectomy, and is further diminished by the partial removal of the other adrenal (66). This speaks well for the sensitivity and the quantitative possibilities of Ingle's test.

The manner in which the adrenal cortex stimulates hepatic gluconeogenesis is by no means clear, but evidence is forthcoming that it influences the mobilization and catabolism of both protein and fat. As regards protein metabolism in normal animals, Babad (5) has reported that in rats maintained on a diet which gave a minimal nitrogen excretion, cortin had no influence on the urinary excretion of nitrogen or creatinine. It did increase the excretion of uric acid and total purine bodies. In rats treated with thyroxin, in which the nitrogen excretion and creatinuria were increased, cortin had no effect (6). However, previous work (83) had shown that nitrogen excretion was decreased following adrenalectomy, and that the administration of cortical extracts restored the nitrogen output to normal. In addition, the increased glycosuria observed after the treatment of adrenalectomized-depancreatized animals with cortical fractions or steroids was accompanied by a corresponding increase in the urinary nitrogen. Wells *et al.* (150, 151, 152) have recently demonstrated similar effects with the cortical substances in phlorizinized adrenalectomized rats.

Concerning the mobilization of fat, it had been shown that the phospholipids and fatty acids of the blood were decreased following adrenalectomy (153), and that various procedures which increased the fat content of the liver in normal animals usually failed to do so in the absence of the adrenals (89, 145). Barnes *et al.* (11) have recently fed spectroscopically active fatty acids to fasting normal and adrenalectomized rats. While they were able to identify the administered fat in the livers of their normal animals, this was not the case in the operated animals. The work of Nelson *et al.* (103) gives an indirect indication of the decreased catabolism of fatty acids after adrenalectomy. They found that the rate of utilization of intravenously injected sodium β -hydroxybutyrate was markedly reduced in adrenalectomized rats, as compared to normal animals. Since adrenalectomy does not change the blood ketone level, it may be inferred that the production of ketones from fatty acids is diminished in the absence of the adrenals.

Finally, it should be noted that, while the effect of the adrenal

cortex on hepatic gluconeogenesis is unquestionable, there is as yet little evidence that this influence is a specific one exerted directly on the liver. The fact that fed, adrenalectomized animals can maintain good carbohydrate levels suggests that the reduced carbohydrate levels of fasting may result from a disability in the mobilization of protein and fat from the peripheral stores. This difficulty, as some authors (103, 152) have suggested, might be aggravated by a general reduction or defect in some basic metabolic function.

THE THYROID

One of the most striking developments of the past few years in regard to thyroid physiology was the demonstration by Ludwig & von Mutzenbecher (85) since confirmed (2, 55), that the iodination of casein gave rise to a material exhibiting the physiologic activity of the thyroid hormone. Hydrolysis of the treated protein yielded thyroxin. Lerman & Salter (79) have now reported similar results, obtained by incubating serum proteins with iodine salts. They have tested their product clinically and have reported its successful use in the treatment of thirteen patients suffering from myxedema. Further evidence that the actual thyroid hormone is probably a protein compound rather than thyroxin itself, is afforded by the work of Canzanelli *et al.* (24). They found that the Q_{0_2} of slices of kidney, testis, and heart muscle from the guinea pig, when tested *in vitro*, was increased by thyroglobulin but not by thyroxin.

The general interest in the functions of the anterior hypophysis has tended to focus attention on the control of thyroid secretion by humoral mechanisms. Indeed, Uotila (143) has recently demonstrated that the administration of thyroxin caused thyroid atrophy while subtotal thyroidectomy was followed by compensatory hyperplasia of the residual tissue, even in rats in which the pituitary stalk had been sectioned. He concluded that the thyrotropic function of the anterior hypophysis was largely controlled by the thyroxin level of the blood, without the mediation of the hypothalamico-hypophyseal nervous pathways. He minimized but did not exclude the influence of nervous stimulation on thyrotropic hormone secretion. Friedgood & Cannon (44) have continued their work on the nervous intermediation of thyroid activity, and have shown that the anastomosis of the right phrenic nerve to the cervical sympathetic resulted in an increased basal metabolic rate,

tachycardia, and restlessness in two out of twenty-eight operated cats. This was accompanied by a right-sided exophthalmos and respiratory hippus in one of the animals. The removal of the right lobe of the thyroid from these animals lowered the oxygen consumption. The severance of the nervous anastomosis returned the basal metabolic rate to within the normal range. Brock *et al.* (21), who stimulated electrically the distal ends of the cut cervical sympathetic trunks in rabbits, obtained an increase in the basal metabolic rate in one out of eight animals. Bilateral cervical sympathectomy more consistently reduced the basal metabolic rate from 10 to 30 per cent in rabbits, and from 20 to 35 per cent in cats.

The influence of hyperthyroidism or of the administration of thyroid substances in increasing protein catabolism and nitrogen excretion has long been recognized. The aggravation of clinical diabetes mellitus by hyperthyroidism, and its amelioration in hypothyroid states, has linked the thyroid activity on protein breakdown with gluconeogenesis from protein. Sternheimer (140) has now shown that the so-called latent period between the injection of thyroxin and the first rise in oxygen consumption is not a period of inactivity. Within six hours after the injection of a single dose of thyroxin into rats, he found a loss of liver glycogen and the beginning of a rise in liver protein. These changes became more marked up to about the forty-eighth hour, and then showed a reversal in direction. By the eighty-fourth hour the liver glycogen reached a peak well above the original control level, while the total nitrogen of the liver, though falling, was still above the original figures. These and other observations indicated that thyroxin first causes a mobilization of protein from the peripheral tissues, and also a proliferation of the liver cells which may be partly at the expense of the initial glycogen stores. Subsequently, there is a new formation of carbohydrate from protein. Gluconeogenesis from protein has also been observed by Wells *et al.* (150, 151, 152) in phlorizinized normal, adrenalectomized, and hypophysectomized rats which were treated with thyroxin or thyrotropic hormone. In agreement with this, the studies of Klein (75) have demonstrated an increase *in vitro* in the *d*-amino acid oxidase activity of the liver of rats fed thyroid substance. This increase did not depend upon the non-protein (flavin) component of the enzyme system, but rather upon the increase in the protein component.

In view of the above findings it is difficult to understand the

relatively minor or negative results which have been obtained either by thyroidectomy of depancreatized animals or by the administration of thyroid substance to such animals. In 1938, Dohan & Lukens (33) reinvestigated the effect of thyroidectomy upon pancreatic diabetes in the cat. The small (though significant) influence which they observed, as compared to the marked effects of hypophysectomy, led them to conclude that the secondary atrophy of the thyroid gland has little to do with the profound modification of diabetes which follows removal of the hypophysis from the depancreatized animal. However, Soskin *et al.* (131) later demonstrated that the administration of thyroxin to hypophysectomized dogs maintained a normal blood sugar level throughout long periods of fasting and increased their urinary nitrogen excretion to that of fasting normal dogs. It is obvious, therefore, that the secondary atrophy of the thyroid gland probably plays an important part in the decreased endogenous protein catabolism and in the related carbohydrate disturbance of the hypophysectomized animal. The question then arises as to why Dohan & Lukens, as well as previous investigators, were not able to demonstrate the role of the thyroid in depancreatized animals. Indeed, they have recently reported on the subject again (86), this time to the effect that partially depancreatized cats given thyroid extract in doses sufficient to produce tachycardia and loss of weight did not exhibit any increase in glycosuria. Anterior pituitary extract readily increased the sugar excretion in the same animals. We had obtained similar (unpublished) results in our laboratory, not only in depancreatized dogs, but also in depancreatized hypophysectomized (Houssay) animals. One might speculate that the thyroid influences gluconeogenesis from protein in the liver by inhibiting the previously mentioned anabolic action of insulin on protein metabolism. If this were so, thyroid hormone might be expected to have little effect in the absence of the pancreas. But such an action of the thyroid would be difficult to reconcile with the report of Johnston & Maroney (71) that small amounts of thyroid are anabolic in effect, as judged by the positive nitrogen balances obtained in growing children. It would also be out of accord with the evidence that the growth hormone of the anterior pituitary gland is more effective in the presence of the thyroid gland than in its absence, and that still greater growth can be obtained when thyroxin is administered along with the growth hormone (41).

It may be that a hormonal activity of the pancreas, other than insulin secretion, is involved in the thyroid action on hepatic gluconeogenesis. The possible role of lipocaic should be investigated, for the relation of the thyroid to lipid metabolism (though long recognized) remains obscure. It has again been recorded that the serum lipids and serum cholesterol are very significantly increased in clinical myxedema (48). Abelin (1) has recently shown that, in rats, thyroid feeding or the injection of thyroxin raises the total cholesterol of the skin as much as 300 per cent, while at the same time the lipid content falls. Thus, the ratio of cholesterol to lipids in the skin rises to eight times the normal figure.

The influence of the thyroid on the metabolism of muscle has been discussed by Wang (146) in the course of an admirable review on creatine metabolism. This author has concluded that the creatinuria of hyperthyroidism in man bears no relation to the oxygen consumption. The excretion of creatine is due to the breakdown of the creatine phosphate of the muscle. In rabbits, the creatine excretion is increased and the creatine and creatine phosphate contents of the muscles are decreased by thyroxin administration. Thyroidectomy produces the reverse effects.

There remain certain unrelated reports on thyroid physiology which deserve mention because of their interest or potential importance. Smith & Perman (125) have again raised the question of the protective action of vitamin A against thyroid hyperactivity or thyroxin administration. They showed that in cats, the administration of carotene with thyroxin caused an average rise in the basal oxygen consumption of 25.4 per cent, as compared with a rise of 45.7 per cent when thyroxin alone was given. Their observation that the carotene did not affect the loss of weight caused by the thyroxin does not seem to be significant in view of the short duration of their experiments and because the quantity of food consumed was not controlled. Their paper contains a good review of the previous work on vitamin A and thyroid action, but they do not mention that fats (the usual carriers of the vitamin) have themselves been shown to exert an antithyroid effect (63, 84, 135).

Brull (23) has demonstrated that the diuretic action of thyroxin is due, in part at least, to a direct action on the kidney rather than to an indirect influence through water mobilization from the body as a whole. He transplanted a kidney from a thyroxinized animal and one from a normal animal into the femoral vessels of a third.

The water output from the previously thyroxinized kidney was much greater than from the contralateral normal transplant. This was especially so when diuresis was stimulated by removal of the posterior lobe of the hypophysis of the animal into which the transplants were made.

Finally, the beginning of the application of radioactive iodine in the study of the thyroid gland is worth noting (54). Hertz *et al.* (62), using this method in rabbits, have now reported that the normal thyroid absorbed about eighty times the amount of administered iodine which might have been expected to enter the gland on the basis of a uniform distribution throughout the body. The hyperplastic thyroid held from three hundred to four hundred times the distribution value. The curves of the uptake of iodine by hyperplastic thyroids differed according to the agent used to produce the hyperplasia. This suggests that there may be functionally different hyperplastic states of the thyroid.

THE ANTERIOR HYPOPHYSIS

Collip (28) has recently reviewed the various separate effects which have been obtained by the administration of anterior pituitary extracts. These include growth stimulating, thyrotropic, gonadotropic, corticotropic, lactogenic, diabetogenic, ketogenic, liver-fat increasing, respiratory quotient lowering, blood-lipid increasing, oxygen-consumption increasing, anti-insulin, anti-epinephrine, glycotropic, glycostatic, and chromatophore expanding actions. There are few who believe that these numerous effects obtained under different conditions of experimentation indicate that there are as many separate hormones secreted by the anterior hypophysis. Collip suggests that as few as two or three separate hormone proteins may account for all the functional activity. The dosage may play a role since, for example, the growth hormone in small doses has only growth effects, while in larger doses it also exerts some corticotropic and lactogenic action. Species differences in the test animals may also be a factor. Anterior pituitary extract will cause a permanent diabetes in dogs but fails to do so in rats (91). There is also the probability that a number of functions listed by Collip are actually duplications of other effects. Thus, Jensen & Grattan (70) have reported that the anti-insulin effect of anterior pituitary extracts is due to the adrenotropic fraction. They found

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that the administration of adrenotropic extract, adrenalcortical extract, corticosterone, and desoxycorticosterone to mice resulted in a significant resistance to the action of insulin, while the injection of thyrotropic extract, prolactin, follicle-stimulating hormone, and thyroxin were without effect. Similarly, it has been found that the diminished absorption of glucose by the intestinal tract after hypophysectomy (109) is probably due to a lack of the thyrotropic hormone, for it may be corrected by treatment with thyroid hormone (115).

There are also complications of another sort in judging the demonstration of a hormone action when extracts are given or a gland is removed. These complications have to do with the more or less incidental reactions of the entire organism to certain nonessential materials contained in the injected gland extracts, or to certain secondary reactions of the organism to the condition promoted by the injection of a hormone or the removal of a gland. Thus Dohan & Lukens (34) have reported that the chronic administration of anterior pituitary extract to depancreatized dogs at first increased and then decreased the severity of the diabetic syndrome. The serum of dogs treated for ten months with anterior pituitary extract, when injected into depancreatized animals, reduced their glycosuria and urinary nitrogen excretion. These results may be likened to the "anti-hormone" effects previously obtained with the gonadotropic fractions of anterior pituitary extract and like them are probably due to nonspecific antibodies formed in response to the proteins contained in the injected extract. The decreased food intake which leads to marked undernutrition following hypophysectomy may also be responsible for some of the results usually attributed specifically to the lack of the pituitary hormones. Mulinos & Pomerantz (102) studied the effects in rats of complete inanition during starvation, and of chronic inanition resulting from an allowance of approximately half the normal food intake. They found that the loss of weight and the histological changes in the endocrine glands resembled those following hypophysectomy. The authors concluded that inanition affected the anterior hypophysis in such a manner as to reduce its secretion of the trophic hormones. It would be interesting to know whether all their results would or would not have been prevented by the injection of anterior pituitary extracts into their chronically undernourished animals.

In view of the attendant difficulties it is not surprising that the

results of attempts at the separation and purification of the various fractions of anterior pituitary extracts continue to be difficult to harmonize and continue to disclose hitherto unsuspected effects. Bergman *et al.* (12) believe they have separated four entities from anterior pituitary extracts, namely, lactogenic, thyrotropic, gonadotropic, and the carbohydrate metabolism factor. Meamber *et al.* (94) have reported that precipitation with cysteine enabled them to separate out the lactogenic and thyrotropic effects from growth fractions of anterior pituitary extract, this procedure resulting in the preparation of almost pure growth hormone. Greaves and co-workers (50) have described the properties of a more purified diabetogenic factor extracted at pH 11. It was non-dialyzable and was destroyed by 100°C. for fifteen minutes at pH 10. This diabetogenic material was ketogenic and lowered the respiratory quotient. It was rich in the growth factor but exhibited little prolactin action. Teague (142) has reinvestigated the association of the melanophore hormone with the "specific metabolic principle of the pituitary" previously reported by Collip and co-workers (15, 108, 111). According to Teague, preparations of the pituitary gland rich in melanophore hormone, obtained from various sources and prepared by different methods, varied considerably in their effect on oxygen consumption in rats. The melanophore activity of extracts could be selectively destroyed without removing the metabolic effects. It was concluded that the melanophore hormone was not identical with a substance in the pituitary extracts which would increase the metabolic rate. It was further pointed out that the results did not support the existence of a specific metabolic principle of the hypophysis, since it was found in the course of the work that metabolic stimulation was produced by a pituitary extract after treatment with acid and after tryptic digestion, and since such metabolic responses were occasionally obtained with extracts of muscle, liver, and kidney. Collip (28, 29) has also reported the action of a pituitary extract which stimulates the "dark" cells of the adrenal medulla, without affecting the chromaffin tissue. The extract is active when administered by mouth. The significance of this action must await enlightenment as to the function of the "dark" cells. Finally, Houchin (64) has been able to decrease the alkali-soluble protein components of the liver with anterior pituitary extract fractions and has suggested the existence of a protein-metabolism hormone which is distinct from the lactogenic, thyro-

tropic, carbohydrate-metabolism, fat-metabolism, and gonadotropic hormones.

The spectacular decrease in the carbohydrate levels of the tissues in the fasting hypophysectomized animal, the restoration of these levels by anterior pituitary extract, and the diabetogenic action of the latter in the normal animal have been interpreted in two ways: either the hypophysis stimulates sugar production by the liver, in which case the effect of hypophysectomy may be ascribed to a decreased gluconeogenesis, while pituitary extracts would accelerate this process; or the hypophysis inhibits the use of sugar by the extrahepatic tissues, in which case its removal would accelerate sugar utilization and the administration of pituitary extracts would depress it. Russell (117) has continued her studies on the disposition of administered carbohydrate in rats and on the influence of the anterior hypophysis on the manner in which the carbohydrate is utilized. She has found that anterior pituitary extract increases the muscle glycogen and depresses the respiratory quotient in normal rats, whether fasted or fed. In adrenalectomized animals anterior pituitary extract fails to increase the muscle glycogen or depress the respiratory quotient and actually depresses the blood sugar level during fasting. The administration of cortin together with anterior pituitary extract to adrenalectomized rats produces results similar to those obtained with anterior pituitary extract in normal animals and, in addition, increases liver glycogen. Russell concluded that anterior pituitary extract diminishes the oxidation of glucose, deposits muscle glycogen, and acts synergistically with cortin in its effects on carbohydrate metabolism. Soskin *et al.* (130, 132) have criticized Russell's interpretations of her data and have offered evidence that anterior pituitary extract does not depress carbohydrate utilization, while hypophysectomy results in a marked reduction in this function. They questioned the validity of Russell's interpretation of the respiratory quotient (127) and pointed out that her assumption that the changes in body carbohydrates in an intact animal over a period of time was an index of the amount of carbohydrate utilized was erroneous because it ignored the unknown amounts of sugar formed by the liver during the same interval. Soskin *et al.* obtained their results on eviscerated normal and hypophysectomized dogs by the chemical balance method previously discussed in the section on the pancreas.

That anterior pituitary extract acts on the liver was shown by

Lee & Freeman (78) who found that the administration of growth hormone causes an increase in the liver weight of rats. The total number of hepatic cells was increased, for the number of cells per gram of liver tissue remained unchanged. However, the influence of anterior pituitary extract on nitrogen metabolism and upon gluconeogenesis from protein in the liver has been obscured by the simultaneous operation of the anabolic effect of insulin which we have previously discussed. Harrison & Long (57) have reported that anterior pituitary extract reduced the urinary nitrogen excretion, the nonprotein nitrogen of the blood and the blood sugar in normal fasting rats, although the ketonuria was increased. These effects were found to persist even after adrenalectomy. Similarly, Gaebler and co-workers have shown that the administration of anterior pituitary extract to normal dogs caused an increase in urine volume, water intake, body weight, and oxygen consumption. It decreased the phosphate and nitrogen excretion in the urine (45). In phlorizinized dogs anterior pituitary extract increased the ketonuria, but still had a tendency to decrease the nitrogen output (46). However, after pancreatectomy the same pituitary extract greatly increased ketonuria, glycosuria, and nitrogen excretion (46). It must be concluded that anterior pituitary extract accelerates protein catabolism and gluconeogenesis from protein by the liver, but that in the presence of the pancreas the effects of the anterior pituitary extract (in the amounts and for the duration of time given by the above workers) were masked by the opposing action of insulin.

With regard to the influence of anterior pituitary extract on the catabolism of fat, recent work (46, 57, 104) has again confirmed its ketogenic action. However, the work of Oastler & Anderson (106) suggests that caution must be exercised in the interpretation of evidence regarding ketosis which is based on examination of the urine alone. They administered epinephrine to rats on a high fat diet in order to increase the ketosis. Hypophysectomy almost completely abolished the ketonuria in these animals, but the blood ketones rose to high values. However, where ketonuria does appear in response to anterior pituitary extract administration, it can apparently be interpreted with safety as an increase in ketone formation. Harrison & Long (58) have shown that the utilization of acetoacetic and β -hydroxybutyric acids in perfused muscle is not impaired by fasting or by administration of phlorizin or anterior pituitary

extracts. That the increased catabolism of fat is accompanied by gluconeogenesis from fatty acids is suggested by the work of Neufeld, Scoggan & Stewart (105). They injected various anterior pituitary extracts as prepared in Collip's laboratory into female mice and made chemical determinations of the entire carcass of their animals. They found an increase in the total glycogen content, a decrease in the amount of fatty acids present, and no change in the nitrogen.

Recent work in which the insulin content of the pancreas of animals has been determined after various procedures has helped to explain some of the effects of anterior pituitary extract. Best and co-workers (14) have shown that the insulin content of the pancreas of rats is diminished by fasting or by the feeding of diets high in fat. In hypophysectomized rats the insulin content of the pancreas is similar to that of normal rats when they are equally well fed (52). Fasting, high fat diets, and the administration of insulin diminish the insulin content of the pancreas, even when the hypophysis is absent. The histological picture of the islets of Langerhans after such treatment suggests that these procedures tend to put the beta cells of the islets "at rest." However, soon after the production by Young of a permanent diabetes in dogs by means of prolonged treatment with large doses of anterior pituitary extract it became known that this pituitary diabetes was accompanied by a progressive destruction of the islet cells of the pancreas. Richardson (112) has made histological studies of the pancreatic glands of such animals and has reported that the islets exhibit a reduction in size, hyalinization, and a degranulation of the beta cells. Best *et al.* (13) have now reported that the pancreas of the dog with pituitary diabetes contains 0 to 0.2 units of insulin per gram as compared with the average figure of 3.4 units per gram in the normal animal. Similar reduction in insulin content was obtained by injection of anterior pituitary extracts for seven days. The cessation of the injections was followed by a restoration of the insulin content in four days. The fact that dogs can be rendered permanently diabetic with anterior pituitary extract, but that this has not been found possible in rats, may be explained in part by the observations of Marks & Young (91). They confirmed the decrease in the pancreatic insulin content in the dog with pituitary diabetes, but found that the administration of anterior pituitary extract to rats increased the amount of insulin in the pancreas. They reported

that in this respect the rabbit behaved like the dog, while the mouse resembled the rat.

It is now possible to attempt a partial reconstruction of the series of events which occur when anterior pituitary extract is administered to the dog or to animals which react in a similar manner. It is probable that the injected material evokes a secretion of insulin from the pancreas. Ham & Haist (53) have reported an increased mitotic activity in the islet tissue of the pancreas, as well as in the thyroid, parathyroid, and adrenal cortical glands following the administration of anterior pituitary extract. Weinstein (148) has confirmed the earlier report of Shpiner & Soskin (124) that the injection of anterior pituitary extract may cause an immediate temporary fall in the blood sugar. The secretion of insulin in response to the anterior pituitary extract injection probably also accounts for the decreased nitrogen excretion (46, 57). However, the continuation of anterior pituitary extract treatment eventually exhausts the insulin-secreting cells of the pancreas and apparently permanently incapacitates them (13, 112). The unopposed actions of anterior pituitary extract then become evident and produce an increase in protein and fat catabolism similar to that occurring when anterior pituitary extract is injected into depancreatized animals (46).

Marks & Young (90) have reviewed their extensive observations on the metabolism of dogs made permanently diabetic by treatment with anterior pituitary extract. Their findings and conclusions are best given in their own concise summary, as follows:

1. Dogs made permanently diabetic by treatment with anterior extract differ most obviously from depancreatized dogs in the following respects:

- (a) Some of these dogs require more insulin for the control of glycosuria than do depancreatized dogs;

- (b) The pituitary-diabetic dogs are able to survive for long periods in good health without insulin therapy, if sufficient utilizable food is given. The intensity of the diabetic condition may vary from animal to animal.

2. Removal of the pancreas from a pituitary-diabetic dog resulted in a slight and possibly not significant fall in insulin requirement. The pancreas contained 2.5 units of insulin, compared with an average figure for nine normal dogs, of comparable weight, of 76 units.

3. On a protein diet, the pituitary-diabetic dogs exhibited hyperglycaemia, a substantial glycosuria and ketonuria, with a D/N quotient of over 3.0 in most instances; on a high-carbohydrate diet, these dogs retained about 15% of the total available carbohydrate in the food; on a diet of beet suet, the blood-sugar level, the glycosuria and ketonuria of these dogs were all diminished, and the sugar

tolerance was increased. In one animal, which tolerated a high-fat diet for over six weeks, the addition of casein to the beef-suet diet diminished sugar-tolerance, but did not increase ketonuria, although substitution of raw meat for casein resulted in a substantial rise in ketonuria. These results support the conclusions of Petren (1924), which were drawn from clinical investigations, that protein (meat-food), and not fat, is particularly concerned in the aetiology of ketonuria.

4. The metabolic rate of the pituitary-diabetic dogs was somewhat above that of control normal animals under similar conditions, but the excess above normal was not so great as was found with depancreatized dogs.

5. As indicated by the hypoglycaemic effectiveness of 5 units of injected insulin, by the Himsworth (1936) glucose-insulin test, and by the de Wesselow-Griffiths (1936) serum test, the pituitary-diabetic dogs do not possess any abnormal degree of insulin insensitivity.

It is concluded that the permanently diabetic condition of our animals may well result from the changes observed in the islets of Langerhans of the pancreas, although these changes are apparently insufficient to account for all the observed facts.

There are two additional items in their paper, not mentioned in the summary, which seem of particular interest. In following up their observation of the ketogenic effect of raw meat as compared with casein in their pituitary-diabetic animals, they found that the residue of raw meat which had been repeatedly extracted with hot water exerted only about one quarter of the ketogenic effect exerted by the original amount of the untreated raw meat. The supplementation of the extracted meat with a concentrate of the hot aqueous extract caused a significant increase in ketonuria. Marks & Young also made a number of comparisons between their results and those obtained by Langfeldt (77) on partially depancreatized animals. One might speculate as to the extent to which the differences between pituitary diabetes and pancreatic diabetes might be caused by the presence, in the former case, of portions of the pancreas which are not responsible for insulin secretion.

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ENDOCRINE ASPECTS OF THE PHYSIOLOGY OF REPRODUCTION

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It is obvious that only parts of a year's progress in this subject can be reviewed in a brief chapter. Since important omissions are unavoidable it has been thought best, in part, to consider more fully those aspects of the subject which were entirely or largely omitted in last year's review and in such cases to include some publications made prior to the past year. It is hoped that the omissions will be less serious and less complete because of incidental reference to some of those topics in this and still other chapters of this volume.

SEX HORMONES

There is little left of the erstwhile view that the estrogenic hormones are specific in their nature and action. They are now known to exist in varied chemical configurations, to have no single or common site of origin (organ or sex), and to have very many and probably still unexplored actions whose range already extends beyond the things which most of us are accustomed to relate to either sex or reproduction. At present the androgens appear to have a more restricted field of action and to retain better some shreds of specificity. Today, however, we are in the midst of extensions of our knowledge in both old and new directions, and some light is being thrown upon the major question of the mechanisms involved in the actions of sex hormones. The increased availability of the sex hormones, their newly found associations with the adrenal, their effective administration in pellet form and their wider clinical use have all contributed to a multiplication of studies conducted with these substances and to some further spreading of their hitherto known actions in the body. In this field, as in most others to be considered in this review, the year has been characterized much less by startling developments than by the output of a prodigious volume of observation and experiment. The field itself widens and deepens and there is some evidence that an increasing proportion of current publication represents critical study.

Adrenal participation in sex and reproduction.—From the adrenals earlier workers obtained adrenosterone, with androgenic properties (Kendall and co-workers; Reichstein), in addition to both estrone and progesterone (Engelhart; Beall & Reichstein). Those basic studies show that at least under certain conditions cortical tissue is a definite source of sex hormones; other current studies have indicated additional adrenal-gonad relationships.

The progestational activity of desoxycorticosterone as tested on a large series of rabbits is reported by Holweg (55), contrary to Hoffmann, to be demonstrable only after pretreatment of the infantile or castrate animal with estrogen; the activity found was about one tenth that of pure progesterone. It is thought improbable that a conversion of desoxycorticosterone to progesterone is a preliminary to activity of this type. Another report (169) notes that desoxycorticosterone resembles progesterone in that it produces progestational proliferation in the endometrium of immature rabbits and also in that it induces estrous receptivity response in spayed guinea pigs. In these tests desoxycorticosterone was one sixth to one tenth as potent as progesterone. Robson (135) found that desoxycorticosterone acetate in 7.5 mg. doses also inhibits the response of the uterine muscle of the rabbit to pituitrin *in vitro* and *in vivo*. Pregnancy in ovariectomized and hypophysectomized mice was maintained to full term by 3 mg. daily for five days beginning at twelve days after mating. This dosage is two or three times greater than the required dose of progesterone. The two compounds were found to be equally effective in the inhibition of the estrous cycle in the mouse and in the inhibition of the action of estradiol on the vagina. In a comparison of the activity of desoxycorticosterone acetate and progesterone in female rats adrenalectomized in two stages, at twenty-five and thirty days, others (32) found that 1 mg. of desoxycorticosterone acetate daily (intramuscularly) was beneficial in prolonging life and maintaining considerable growth, while 0.25 or 0.5 mg. daily also had a noticeable effect; 1 mg. daily of progesterone gave a curve for bodily growth that could not be distinguished from that obtained under similar conditions with desoxycorticosterone acetate.

The ability of desoxycorticosterone acetate to exert androgenic action was tested in the capon, mouse, and rat (56). The quantitative results obtained led to the conclusion that the androgenic activity of the compound resembles androsterone more than

testosterone since it is relatively more active upon the comb than upon the rodent accessories. In castrate or adrenalectomized-castrate young male rats, aged twenty-one or thirty-one days, daily dosage of 1 to 10 mg. desoxycorticosterone acetate was found (47) incapable of preventing regression of the ventral prostates; accordingly, the authors conclude that this substance is not androgenic in the rat.

On the other hand, there is evidence that desoxycorticosterone acetate is estrogenic in the human female (142). Typical estrogenic effects were obtained with desoxycorticosterone acetate in the vaginal smears of women past the menopause. On the basis of these observations, it is suggested that the estrogens excreted after the menopause or after surgical castration probably have their origin in the adrenal cortex. This would explain the persistence of estrogen effects in the vaginal mucosa and vaginal smears after surgical castration and possibly also account for individual variations in the severity of symptoms experienced at the menopause or after surgical castration.

It is now known that the prostatic lobes of young female rats react in the same way to injected sex hormones as do the ventral lobes of males, whose glands afforded a quite sensitive test for at least some androgens. Price (110) has supplied evidence that in a certain proportion of female rats the prostate undergoes normal development, differentiation, a secretory phase, and regression comparable to these states in prostates of young castrated males, and also suggests that the source of this temporary stimulation is the juvenile adrenal cortex. Confirming the view that the adrenal participates in these processes, Burrill & Greene (17) found castration to cause complete atrophy in the ventral prostates of 26-, 31-, 36-, and 41-day-old adrenalectomized, castrated male rats. The ventral prostates were partially maintained in castrated, non-adrenalectomized animals at twenty-six and thirty-one days, but not at thirty-six and forty-one days. In the presence of the testes adrenalectomy alone did not modify the ventral prostates at any of these ages. Thus the adrenals appear to have an andromimetic capacity in the younger stages which is lost in the older but still immature animals.

Contributions have been made to still other aspects of this problem. In month-old male adrenalectomized rats the average survival time was markedly decreased by injection twice daily of

androgens (urinary androgenic extract, 50, 150, and 250 I.U.; androstenedione, 5, 10, and 30 I.U.; testosterone acetate, 14 I.U.; testosterone propionate, 50 and 125 I.U. per day); this decrease in survival time was roughly proportional to the unitage used. At 250 I.U. (urinary extract) the survival time was decreased from 7.1 days to 2.7 days (155). Higher doses, 2 mg. daily for ten days, of estrone caused enlargement of adrenals of young pigeons, while 0.1 mg. daily for three days given to young rats yielded clear histological evidence of cortical stimulation (94); further, stilbestrol, 40 to 90 μ g. daily for twenty-eight days, enlarged the adrenals of rats (104). Ovariectomy in mice was found (60) to have only a slight stimulating effect on the growth of the adrenal X-zone, though pregnancy is associated with its rapid degeneration and disappearance, and this same effect is produced by the injection of androgens. But progesterone treatment, up to 5 mg. daily, does not produce any marked degeneration of the X-zone, and hence does not reproduce the effect of pregnancy on the adrenal. These observers further find that progesterone can produce marked stimulation of uterine growth in ovariectomized mice without preliminary treatment with estrogens.

Effects of steroids on the anterior pituitary.—Deanesly (25) investigated effects of estrone and estradiol by implants of compressed tablets of pure hormone under the skin of male rats and mice for periods of 10 to 270 days. This treatment, like continued injection of estrogens, inhibited some of the functions of the pituitary gland. Besides a marked depression of the growth rate, which in these cases need not be considered as evidence of changed pituitary function, there was loss of fertility, shrinkage of gonads and accessory organs, and enlargement of the adrenals and pituitary gland. After removal of the implants these changes were reversed. Similar results were reported (106) from the use of tablets or crystals of still other estrogens. In these tests it was noted further that, in female rats thus treated for three weeks, the injection of pregnancy urine caused an ovarian response similar to that seen in the intact immature rat. After eighteen weeks, however, pregnancy urine produced an ovarian response similar to that observed in hypophysectomized rats. The gonadotrophic content of adult rat pituitaries following castration was observed (73) to reach a maximum after sixty days and an equivalent high quantity was present at one hundred and twenty days.

Weil & Zondek (173) studied the pituitary histology in rats after high and prolonged (nine to sixty-three weeks) dosage of estradiol benzoate and obtained glands with a maximum weight of 100 mg. In such glands the intertubular cleft was markedly widened and in some cases the vascularity was so greatly increased as to lead to intratubular hemorrhages and death. Polygonal and rounded eosinophiles and basophiles were swollen, their Golgi apparatus were maximally dilated, and most of the chromophiles were degranulated. Zondek (181) has reported results from prolonged administration of estrogen on the anterior pituitary (and uterus) of human beings. With a dose of at least 70,000 I.U. the normal ovarian cycle was inhibited and menstruation postponed for from seven to seventy days. Protracted administration of immense doses (6,000,000 I.U. of estradiol benzoate over sixty days) prevents the ripening of the follicle as well as corpus luteum formation; the ovaries resemble those of an old woman and functional castration was accomplished. The reaction of the anterior pituitary of a woman given the last-named dosage was a marked increase in the number of eosinophilic cells—an eosinophilic hyperplasia ("adenoma") forming half of the entire anterior lobe. The other endocrine glands are said to have showed no deviation from the normal.

Earlier work has indicated the presence of increased amounts of gonadotrophic hormone in the pituitaries of women after the menopause and that fact has supplied a reason for measurements of effects of estrogens on both the urinary excretion and the content of gonadotrophic substance in the pituitary of women after castration or the menopause. Observed effects of male and female sex hormones on pituitary gonadotrophic function in women (140) supplements earlier evidence that both types of sex hormone decrease the output of gonadotrophic substance by the human pituitary. In five of seven women treated after the menopause with estrone (10,000 I.U. three times weekly) the urinary excretion of gonadotrophic hormones was reduced to normal limits. Three of four cases responded in a similar manner to the administration of testosterone propionate in doses of 25 mg. three times weekly. In the two cases apparently refractory to the estrogen a definite decrease of urinary gonadotrophic substance was produced by administration of the androgen. Heller & Heller (52) used lower dosage (amniotin, 8,000 I.U. daily orally; 10,000 I.U. intramuscu-

larly in two cases) and obtained no effect on the amount of gonadotrophic substance excreted. Rowlands & Sharpey-Schaffer (141) studied five cases injected daily intramuscularly with estradiol benzoate (10 mg.; 100,000 I.B.U. daily for three to fifty-four days) in oil solution. Urinary excretion was followed in only one ovariectomized woman whose urine contained a small amount of gonadotrophic substance prior to treatment; the injections abolished that small amount. Assays, made on hypophysectomized rats, of the gonadotrophic content of each of these five human pituitaries indicated that estrogenic treatment had decreased both their luteinizing and follicle-stimulating substances.

In an evaluation of these several results one is warranted in recalling that on histological grounds Severinghaus (149) regards the action of estrogens on the pituitary gland as one of stimulation and release of secretion. It is possible that present measurements of urinary gonadotrophin do not correctly reflect the output of gonadotrophic hormone by the pituitary.

An investigation (8) of the action of progesterone on the gonadotrophic activity of the pituitary showed that 4 mg. of progesterone injected daily during three or four days into normal adult rats effectively prevented the induction of pseudopregnancy from cervical stimulation. One tenth to 1 mg. progesterone injected into immature rats together with follicle stimulating hormone for four to ten days decreased the ovarian weight response and inhibited luteinization; in hypophysectomized immature rats, however, no inhibition was obtained. The authors therefore conclude that progesterone suppresses the release of luteinizing hormone from the pituitary and that this suppression is responsible for the absence of estrous cycles during periods of active luteal function. This interpretation requires, as noted by the authors, the further conclusion that some pituitary hormone other than luteinizing hormone is responsible for the continued function of corpora lutea already formed. The question of which hormone is responsible for luteal function (and the suppression of cycles) is thus widely reopened. Lahr & Riddle (70) had earlier found that prolactin suppressed the cycles in rats and that the corpora lutea of such prolactin-treated rats appeared to be in fully functional condition; further Evans, Simpson & Turpienen (36) had also briefly noted that this corpora maintenance factor was present in their adrenotropic and lactogenic preparations. Possible relations of steroid

hormones to these processes is discussed under a subsequent heading.

Effects of testosterone propionate on the pituitary of rats have again been examined. Immature female rats which received daily injections of from 2 to 8 mg. daily for periods of ten or thirty days had pituitary weights slightly less than that of their controls (177). The granules of the basophiles were caused to disappear but the eosinophiles showed no noteworthy change. A second series of female rats was treated for three months at four levels (0.1 to 1 mg.) of injection. Litter-mate sisters and brothers were used as controls. Judged in relation to body weight, the weight of the pituitaries and the adrenals of the injected females were more comparable to those of their untreated brothers than to those of their untreated sisters. An increase in the levels of eosinophiles and granular basophiles occurred in the anterior pituitaries of the injected females, so yielding a cellular pattern in this gland which closely approached that of their untreated brothers and which diverged widely from that of the control sisters. This masculinizing effect was most pronounced in the females receiving the highest amounts of androgen. Reece & Mixner (117) injected 200 μ g. daily for fifteen days into spayed sexually mature rats. The pituitaries of treated rats showed no change in weight but their prolactin content seemed to have increased about forty per cent.

Notable results were obtained by Tobin (164) from destruction of the adrenals of seventeen-day rat embryos (which survived to term) with study of resulting changes in the organs of reproduction. The pituitaries of embryos thus operated showed fewer acidophile cells, reduction in the granulation of the basophiles, and an increase in number of chromophobes, with retardation of differentiation of all three cell types. The thymus glands showed more advanced corticomedullary differentiation and greater number of reticular cells. The parathyroids were of larger size. The growth of ovaries, testes, and accessory organs was not inhibited. These findings suggest that loss of cortical tissue and steroids produced by it were responsible for these changes in the pituitary, thymus, and parathyroids of the embryonic rat.

Actions of steroids on more typical reproductive processes.—Korenchevsky, Hall & Burbank (67) report an informative study on the many effects of prolonged (three and one half months) administration of three androgens and of estradiol propionate, singly

or associated with an androgen, on various visceral and reproductive organs of normal and castrate female rats. Estradiol alone restored the vagina of castrates to normal or supernormal weight and normal (usually estrus) structure; not even a weekly dose of 0.2 mg., however, was able to produce normal size and weight of the uterus, although this dose caused pronounced pathological changes in its structure. There was a co-operative effect between estradiol and androsterone or transdehydroandrosterone on the weight and size of the sex organs, but at the same time the number of rats affected with squamous metaplasia of the uterine epithelium (first step of "precancerous" estrogenic effect) was increased and this condition became more severe. Testosterone propionate produced pronounced progestational changes in the uterus and less strong ones in the vagina. Testosterone propionate had a co-operative effect with estrogen on the weight and size of the uterus, but the occurrence and degree of epithelial squamous metaplasia in this organ were about the same as with the female hormone alone. When, however, progesterone was added to the combination of male and female hormones, or when a large dose of testosterone propionate was injected with estradiol, these "precancerous" changes in the uterine epithelium were prevented to a remarkable degree. Male hormones caused decrease in the weight and size of the adrenals of females similar to that previously described in male rats, while the effect of the female hormone was characterized by peculiar pathological changes. In most cases simultaneous injection of male and female hormones led to enlargement of the adrenals. The tumor-like hyperplasia of the hypophysis produced by the estrogens was measurably neutralized by male hormones. When injected simultaneously, the male hormone more or less completely neutralized the effect of the estrogen on liver, kidneys, heart, spleen, fat deposition, and body weight.

A group of spayed female rhesus monkeys was subjected to a maximum of ten daily injections of estrone by Worthington & Allen (178) and sacrificed individually at varying times during and following the hormone treatment. The rate of mitosis after colchicine was determined in the uterus, tubes, and vagina of these monkeys. Among their several findings the item of chief interest to the present review is the observation that growth and cell proliferation in the genital tract was greatest in the first half of the period of treatment, that it declined with further treatment, and

declined still more upon withdrawal of hormone. It thus appears that even in a most usual or typical action of an estrogen we may observe a relatively early period at which its effectiveness declines. This will again be encountered in effects (such as calcemia and lipemia) which are currently regarded as less typical estrogenic actions.

Additional evidence that exocrine activity of the testis may be supported by androgens was briefly reported by Nelson (100). The testes of rats whose treatment was started immediately (but not otherwise) after hypophysectomy with 2 mg. daily of either testosterone propionate or androstenedione maintained spermatogenesis for at least six months. Though the treated testes became smaller (1.609 gm.) they greatly exceeded the weights of their untreated control (0.273 gm.). Treatment of such rats with adrenal cortical extract (1 to 2 cc. daily) also had an effect observable in the fact that, although it failed to support spermatogenesis, its use for twenty days following hypophysectomy did thereafter enable androgen to reinitiate spermatogenesis in eight of ten tests. The ability of the synthetic estrogen diethylstilbestrol to increase markedly the weights of ovaries of hypophysectomized immature (40 to 50 gm.) rats has been briefly but rather convincingly reported (175).

It has been found (26) that adrenalectomy in the rat rendered its vagina more sensitive to estradiol benzoate, and further that this increased sensitivity is neutralized or corrected by the injection of 0.5 mg. daily of desoxycorticosterone acetate. Selye (145) observed that in spayed rats, not pretreated with estrogen, huge doses (15 mg. daily) of progesterone produce gestational changes in the endometrium, vaginal mucification, and mammary gland development similar to that seen in late pregnancy. Selye believes, contrary to current views, that progesterone can exert all its characteristic actions in spayed females without sensitization by estrogens.

The abnormal clitoris of the rat produced in prenatal or early postnatal life by excessive estrogen administration has been further studied and probably correctly interpreted. Since these changes resemble those produced by androgens, Turner & Burkhardt (166) consider their production by estrogen an andromimetic effect of estrogen. A subsequent study by Greene, Burrill & Ivy (48) makes it more probable that these defects produced by estrogenic treat-

ment are essentially hypospadias and ascribable to inhibition of normal development. This condition is similar to that found in the full-term fetus, and it also closely resembles that produced in males by androgens in which case also it is regarded as arrested development. Stilbestrol was administered by Lisser (82) to a girl aged nearly seventeen years who had never menstruated, who completely lacked breast development and who had an enlarged clitoris and masculine hypertrichosis. Normal breast growth resulted promptly, her first menstrual period occurred after five months, the clitoris atrophied, and the abnormal hair paled and partially disappeared.

Several investigations deal with the ability of sex hormones and adrenal steroids to substitute for progesterone in the maintenance of pregnancy or to sustain a functional corpus luteum. In the rat it was observed that progesterone (one or two rabbit units daily) will maintain pregnancy to the twenty-first day when castration is performed on the fourth day (139). Testosterone (20 mg.), testosterone propionate (5 to 15 mg.) and androstenedione (10 to 15 mg.) maintained pregnancy in rats only when castration was performed during the last half of pregnancy (46). Hartman (50) showed the dispensability of the corpus luteum in the rhesus monkey after the first month of pregnancy. The relation of progesterone and estrogen to the maintenance of pregnancy in the castrated rabbit has been studied effectively. Aided by preparations of pure progesterone Allen & Heckel (3) reversed their conclusion reported two years earlier and in agreement with others now find it possible with progesterone alone (four rabbit units daily) to maintain pregnancy in the rabbit in a normal manner after castration on the eleventh day from mating, i.e., after implantation of the embryos has occurred. In the hypophysectomized rabbit Robson (133) obtained evidence that the favorable action of estradiol on the corpus luteum of pseudopregnancy is antagonized by neither progesterone nor testosterone. In cases of normal pregnancy (134), with hypophysectomy on the twenty-first day after mating, partial success was obtained in maintaining pregnancy by injection of 2 μ g. of estradiol benzoate daily from the twenty-first to the twenty-eighth days. Under this treatment immediate abortion was prevented, some live fetuses were born, the corpora lutea were apparently functional, and the mammary glands weighed appreciably less than normal glands. Robson (136) later found

that in rabbits hypophysectomized on the twenty-eighth to twenty-ninth days after mating pregnancy was prolonged by administration of 1.9 to 2.5 mg. progesterone per day, by 2 μ g. estradiol benzoate per day, and also by the synthetic estrogen triphenylethylene. This action of estrogen was considered very probably due to a maintenance of the activity of the corpus luteum. It may be noted that other earlier studies which provided evidence that the placenta exerts a favorable action on luteal function are at least not inconsistent with Robson's view that estrogen is chiefly involved. However, as noted in the previous section, the role of the hypophysis is not yet clear and additional work seems necessary before the regulation of luteal function is satisfactorily known.

The extensive clinical use of the sex hormones is contributing valuable information concerning the physiological action of these substances. It may here be noted that testosterone propionate appears to have been used successfully in certain cases diagnosed as male climacteric or involutional melancholia (163), in adult male hypogonadism and benign prostatic atrophy (93, 167), for inhibition of ovarian activity (43), and for reducing excessive milk secretion (13b, 68). Estrogens have been used in the medical induction of labor (34, 97). Estrogens, including stilbestrol, also relieve symptoms of the menopause (39). Frequent oral doses (0.25 to 4 mg.) of estradiol were used to suppress excessive milk flow (74). It is reported (176) that, in the absence of estrogenic hormone, progesterone has little effect on the human myometrium; also, that testosterone propionate alone produces uterine contractions resembling those seen after combined estrogen-progesterone treatment and simulating those of the luteal phase of the menstrual cycle.

Sex hormones and calcium metabolism.—Advance in this peculiarly confused subject is difficult to discuss and understand apart from an historical statement. It is now fourteen years since it was demonstrated by Riddle & Reinhart that the serum calcium of the female pigeon is regularly and normally increased by more than 100 per cent on each recurrent occasion on which her ovary functions. A year later it was pointed out (127) that coincident with this calcemia, and with the production of each pair of rapidly grown yolks, the bird's ovary releases estrogenic hormone in the huge quantity required to enlarge the bird's oviduct by 1,000 per cent. Four years ago Riddle & Dotti (129) showed the uniform

ability of synthetic estrogens to increase (up to 400 per cent) the serum calcium of normal male and female doves and pigeons, and of hypophysectomized and hypophysectomized-castrate pigeons of differing age and sex. Slight effects which were considered significant were also then partially published for rats and dogs; the tests then reported indicated no effect in rabbits. In none of these cases was the calcium increased by androgens in doses of 3.5 to 30 capon units daily for seven days.

All of a dozen subsequent studies confirmed the finding that egg production in birds, and also in amphibia (Charles, Hogben, Zwarenstein & Shapiro), is associated with an increase of 100 to 200 per cent in blood calcium. All reports for birds, with two exceptions to be examined, are in agreement that the administration of estrogenic hormone produces calcemia. Marlow & Koch (89) properly consider the observed fluctuations in their control or un-injected values a bar to attributing significance to the values obtained from estrogen dosage in their tests; and the normal variation found in the cockerels used by them is both unusual and perplexing. But there are three recognizable reasons for the failure of their study to demonstrate the action of estrogen in fowl. (a) The age and condition of the female birds used was evidently not so known and checked as to permit an adequate test with the low dosage of estrogen employed. If estrone production in the mature bird's own ovary greatly changes her blood calcium it is essential that all females used be so young—and the age at beginning maturity varies with different breeds—that none of them will have initiated this estrogen production, neither at the beginning nor by the end of the series of tests. (b) The estrogen dosage was insufficient. (c) Finally, dosage for only two, three, or at most four days, with omission on each third, fourth, or fifth day, does not provide the continuous dosage which other studies yielding positive results have used and found necessary. In most cases the daily dosage used by Marlow & Koch is not made clear. For six birds this is said to be "5, 10, and 15 R.U. per 100 gm. body weight"; for six other birds the apparent total is 10 R.U. daily, and for six others a total of 200 R.U. daily. The preparation used throughout on fowl was described not as pure estrogen but "an estrogenic concentrate (UE) from human pregnancy urine."

The recent report of Marlow & Richert (90) is chiefly concerned with the failure of the very small amounts (about ten rat units

daily) of estrogen extractable from a few egg yolks or fowl ovaries to affect the blood calcium of pullets. This failure is therefore of no consequence to the point under consideration, though it does question the validity of the report (4) that intraperitoneal injections of egg yolk induce calcemia in fowl. It is nevertheless notable that the dosage of estrogen necessary to increase experimentally the serum calcium of the bird is relatively large but apparently only of about the same order as that used by the female bird herself to evoke an equivalent calcemia. In both cases the extent to which the weight of the bird's oviduct is increased provides a measure of the amount of estrogen required for its development along with concurrent calcemia, and that amount is several thousand rat units of estrogen continuously administered during several days. A recent study (10) confirms earlier positive results, and also indicates that peaks and partial declines in the calcium level of the fowl may occur under continuous dosage, as was earlier reported for dogs (21, 130).

From another source some confusion, and indeed a point of embarrassing uncertainty, enters into this question. Apparently, estrogen—or at any rate a certain excess of estrogen—induces calcemia through action on the parathyroid glands; but some difficulty attends all attempts definitely to establish that fact, and there have been far too few attempts. In most animals complete parathyroidectomy also requires thyroidectomy and the latter provides complications since the thyroid and several other organs and conditions affect either the ingestion, absorption, mobilization, utilization, or excretion of calcium. To this point the calcemia produced by estrogens has been considered from the standpoint of its bearing on the question of whether estrogens do or do not show this action. The larger question of which this is only a part is whether estrogens have a special role in calcium metabolism. From this broader aspect of the problem it is clear that in mammals estrogens may be able to bring about marked changes in bone while altering the blood calcium but little. The bird of either sex can withstand an increase of 500, or perhaps 1,000 per cent, in its serum calcium without clear evidence of inconvenience. Mammals apparently can not do so, since one eighth of a 500 per cent increase has been observed to cause lethargy and inhibition of activity in the higher nervous centers of dogs (5). The early and temporary nature of the response in dogs, and the probable value of increasing

the estrogenic dosage daily in order to increase the blood calcium are significant observations (21). Mammals clearly resist (79, 89, 129) an increase in their blood calcium by estrogen with better success than do birds; but certain available data for effects of estrogens on coagulation time in mammalian blood (143) and recent data for bone changes in estrogen-treated mammals (noted below) reinforce results obtained in many of the direct measurements (21, 129, 130) of the blood calcium change, making it wholly probable that qualitatively the effect of estrogens is similar in amphibia (187), birds, and mammals.

Prolonged dosage with pituitary gonadotrophic substances increases the serum calcium of adult hypophysectomized female pigeons, but not of adult or immature male pigeons; prolactin, cortin, and androgens all failed to increase the serum calcium even in normal pigeons (129, 130). Riddle & Dotti think these results demonstrate that estrogen produced in the bird's ovary is a necessary link in the chain which connects the pituitary with calcemia—and therefore probably with changes in the parathyroids and bone. It was briefly reported (131) that estrone is wholly incapable of increasing the serum calcium of parathyroidectomized rats though its effect is measurable in normal rats. These and other studies to be noted immediately definitely pose the question whether the parathyroid glands are controlled directly by the gonads (and adrenal cortex) and only indirectly by pituitary hormone. And, if so, does it follow that the parathyroids are essentially a part of the reproductive system? It would seem, at least in the bird, that the pituitary influence on bone growth is describable, in terms of the following sequence of activating agents: gonadotrophic hormone→steroid (estrogenic) hormones→parathyroid hormone→osteoblast and osteoclast activity. These considerations assume greater importance because of the fact that available data do not satisfactorily show that the pituitary produces another hormone which acts directly on the parathyroids.

In fowl chronically treated with estrogen, with consequent dwarfism, Zondek (180) noted a marked hypercalcification of the bones. Pfeiffer & Gardner (109) reported a careful study of changes in bone and serum calcium induced by estrogen in male and female pigeons, and also occurring normally at periods of ovulation in female pigeons. Hypercalcification in the marrow-containing bones was observed under both conditions; it was marked in males

treated for two weeks with 1,000 I.U. daily and reached a maximum after five weeks. Another study (71) extended this inquiry to fowl with quite comparable results. Maximum bone change was produced within three weeks by 10,000 I.U. estradiol daily, and a maximum serum calcium value of 78.0 mg. per cent was recorded. A study of seasonal hyperossification changes in the English sparrow provided less clear evidence of the decisive role of estrogens, since such changes are also pronounced in male sparrows at the height of the breeding season.

Certain studies have supplied clear evidence that estrogens exert a like action on the bones of mammals, and provide still other items of interest. The original observation of Tausk & de Fremery (161) in 1935 that the epiphyses unite more rapidly in dogs receiving estrogen seems to have stimulated little further study of this subject during the next two years. In Copenhagen Seeman (144) found a fairly high estrogen content in boar testes (234 M.U. per pair), but none in testes of bulls and rams. When estrogen-containing lipid extracts of the testes of boar (but not of bull and ram) were injected into infantile male and female guinea pigs, they markedly altered the growth of their bones, producing hypercalcification and earlier closing of epiphyseal lines in long bones, often with slight reduction of body weight as compared with un-injected controls. Seeman thinks that the closure of the epiphyses which begins at puberty may be causally associated with the presence and action of estrogen in both males and females. Talbot (160) administered estradiol to immature rats for seven to nine days after birth and noted a significant advance in the skeletal age of the females but not of the males. Gardner & Pfeiffer (41) found that prolonged dosage of mice with estrogens results in nearly or quite complete replacement of the marrow cavities of the femurs of mice with endosteal bone, and indeed that comparable changes occurred throughout the skeleton except in the immediate vicinity of the pubic symphysis. They later observed (42) that testosterone propionate inhibits both the pubic resorption and hypercalcification which occur in mice under prolonged estrogen administration.

Another study (174) was concerned with the composition of bones of mice after dosage with estrogens or androgens. Large doses of estradiol benzoate were found to produce significantly higher concentrations of inorganic substances in the femurs and pelves than in control mice or mice receiving testosterone pro-

pionate or estradiol benzoate plus testosterone propionate. The ratio of calcium to phosphorus in the femurs, but not in the pelvis, of mice receiving estradiol benzoate is significantly higher than in the other groups. A higher ash content of bones of untreated females as compared with untreated males was observed and cited as reflecting the influence of the animal's own hormones on its skeletal growth.

Suitable studies of the phosphatase activity of the blood serum of animals or subjects treated with estrogens or androgens may be expected to supply important information concerning osseous changes in man and mammals. Though there is poor accord of opinion on the subject, a recent study (171) reports an increased phosphatase activity in a majority of forty-five patients with chronic nonspecific arthritis. The administration of adrenal cortical extract (but not desoxycorticosterone acetate) was followed by a reduction of phosphatase activity and symptomatic relief. This instance serves as a reminder that some earlier investigations support the view that the adrenals exert an influence on the parathyroids and osseous metabolism.

It is clear that gonadotrophic pituitary extracts should affect bone growth if the estrogen-secreting organs, and probably the parathyroids, are left intact in the subjects injected with such extracts. Thus the administration of an acid extract of beef pituitary (which surely contained much gonadotrophic hormone) was observed (152) to stimulate proliferation and ossification of cartilage in guinea pigs, and here too the balance between proliferation and ossification could be disturbed in favor of one or the other of these two processes. It is obvious that the adequate interpretation of proliferation and ossification following the administration of any crude anterior pituitary requires the use of suitably modified animals in addition to normal ones.

The action of sex steroids on blood fat.—Despite great gaps in our knowledge, particularly in the case of mammals, it is of much importance that estrogenic hormones have now been shown to have surprising ability to increase the blood fat in birds. An added significance of this action of estrogens is provided by the fact that in female birds a physiological lipemia serves a primary and indispensable reproductive need and that androgens have no similar action. Even before obtaining a much needed clarification of the action or lack of action of these steroids on the fat metabolism of

mammals, it can be said that in birds estrogens have now been shown to exert a specific action on the blood fat of both sexes, and under quite normal conditions this action in these animals is intimately concerned with reproduction. It seems well to review the studies of the past two or three years which lead to these conclusions.

Indications of a relation of estrogen to lipemia in birds dates from 1916 and 1927, and many less definite indications that the mammalian ovary has a special relation to fat metabolism are still more ancient. However, it remained for Lorenz, Entenman & Chaikoff in 1938 definitely to prove this relationship in the fowl. (a) They showed rather more satisfactorily than had been done before the amazingly high levels attained by lipids in the blood of fowl in the active laying state (86). This active state of the ovary was also accompanied by a marked increase of liver fat (84). Even on low fat diet, lipemias of 4,719 mg. per cent were found after estrogen (as compared with 389 to 649 mg. per cent in immature control birds). Neutral fat, phospholipids, and free cholesterol all shared in this increase. (b) The lipid content of the blood was shown to be doubled by prolonged stimulation of the ovaries of immature birds with mare serum (35). (c) An estrogenic concentrate (25,000 R.U. per cc.) from pregnant mare serum, given in a total dose 1,000 or 2,000 R.U. to pullets (seventy-four days old) or cockerels (sixty-six days old) practically doubled the amount of blood lipids after twelve to twenty-four hours (not at three or six hours). All lipid constituents of the blood shared in this increase (85).

Nearly simultaneous with the last-noted study were reports by Zondek & Marx showing extreme increases (to 8,000 mg. per cent) of blood lipids of cocks treated with heavy doses of estrone, estradiol (182), and diethylstilbestrol (183). Total liver fat was increased to 25 per cent of the total dry weight. Heavy dosage with progesterone for two days gave a negligible increase of blood fat, while testosterone propionate or acetate probably had no effect. In rabbits, rats, and man high dosage with estradiol benzoate, in part tested up to six days, produced no definite change in blood fat. The study (182) on cocks involved prolonged and heavy dosage, and ability of estrogens to increase blood fat was there for the first time associated with their ability to increase the blood calcium. In thirteen cocks with an average lipemia of 3,600 mg. per cent,

the calcemia averaged 55 mg. per cent (one case of 117 mg. per cent). This association of calcemia and lipemia in cocks was confirmed by others (71) who further showed that after adequate periods the calcemia was partly related to the bone changes already noted in the preceding section. In nonlaying hens given a single injection of gonadotrophic substance (Antex Leo, 240 M.U.) Laskowski (72) obtained a progressive and very marked increase (lasting until the sixth day) in the lipid phosphorus of the blood, followed by decrease to normal on the tenth day. Injections of testosterone propionate into nine schizophrenic subjects during a period of three weeks is reported (112) to have produced a continuous rise in serum lipids followed by a return toward the initial level after medication was stopped.

The above-named studies do not permit a decision as to whether lipemia represents a direct action of estrogen or whether this is mediated by another organ. Contributing to this point, Riddle & Senum (132) have briefly reported results showing that estrone and dihydroestrone exert their full action in immature pigeons of either sex deprived of both anterior and posterior pituitary lobes, and also in pigeons of either sex completely deprived of the pancreas. Large and prolonged dosage with androgens produced no lipemia in either normal or hypophysectomized pigeons, while desoxycorticosterone acetate also has no clear or unquestionable effect. Here too in doves and pigeons, but not in rabbits, it was found that the lipemia induced by pituitary extracts is traceable to their gonadotrophic hormone acting through estrogen produced in the ovary; such gonadotrophic hormone is effective in females only, and it is there effective in the absence of the pituitary. In prolonged tests with both gonadotrophic substances (108 days) and estrogen these observers met with unexplained alternating periods of effectiveness and ineffectiveness of the hormone in maintaining the lipemia (and calcemia), quite as noted for calcemia (10, 21) in the previous section.

These several studies are in full accord with older reports (127) showing that blood fat and lipid phosphorus begin to increase in close coincidence with beginning rapid deposition of yolk (including fat and lecithin) in the pigeon's egg, and also coincident with a temporary growth stimulation of the bird's oviduct, a fact which proved that all these processes were coincident with release of estrogen by the ovary. These several associations clearly indi-

cate that the striking estrogen-controlled lipemias of birds are a normal and necessary aspect of reproduction in these animals. This fairly clear outline of the way in which the pituitary and ovary produce a temporary and quite physiological lipemia in birds should at least prove helpful to the further analysis of lipemias of pituitary origin in mammals.

Mechanism of action and miscellaneous effects of sex hormones.—Information concerning the ability of estrogens to induce peripheral hyperemia as a secondary result of their acetylcholine-liberating properties represents a notable achievement. This particular generalized action of the estrogens not only advances our understanding of the mechanism of their action but it perhaps has special possibilities as a means of bringing other generalized actions of estrogens, such as calcemia and lipemia into a single picture.

In suitably prepared rabbits Reynolds (123) found that the initial hyperemia of the uterus which follows the injection of a mixture of estrogens (Amniotin) is accompanied by an increase in its acetylcholine content. However, this cholinergic action of estrogen was of very transient nature, being highest at one hour and progressively lower at six and twelve hours. It was shown that the vascular changes definitely precede the onset of intermittent estrous motility which begins only at ten or more hours after estrogen administration and that they coincide with the onset of a rapid increase in oxygen consumption earlier noted in such uterine tissues. According to Reynolds, it thus appears that acetylcholine is a vasodilator in a tissue which is quiescent, which has a low metabolism, and in which the blood vessels are of small calibre and therefore offer considerable resistance to the rapid flow of blood through the tissue. This study was repeated and confirmed using pure estradiol and estradiol benzoate. Triphenylethylene was found to be strongly cholinergic on the uterus, but stilbestrol was inactive (124). The fact that stilbestrol is strongly estrogenic, but not appreciably cholinergic, clearly indicates that the estrous response of the uterus does not depend upon the initial cholinergic effect of the estrogens. In transilluminated ears of ovariectomized rabbits, pure estrogens were observed to dilate capillaries and venules, this effect commencing within three to five minutes and attaining a maximum in six to twenty minutes. This dilatation exceeded the period (two and one half hours) of observation (126). In two thirds of twenty human males, estrogens increased finger

volume with no rise in skin temperature. This change persisted for more than two hours and the rate of blood flow was apparently not affected (125).

Zuckerman and associates noted that estradiol in oil induces a rapid change in the water content of many or most organs of the immature rat. In the skin the total amount of water shift was reported to amount to almost one per cent of the body weight (186). Adequate amounts of vasopressin, estrone, progesterone, and testosterone propionate all cause a significant increase in the body weight of axolotls, which is attributed to an increase in body water. With cortin and desoxycorticosterone acetate the results were somewhat variable but tended toward reduction of body weight (29). In mature pig-tailed monkeys large doses of estrogen injected daily during the postovulation phase of the cycle led to swelling in the sexual skin with accumulation of water. The fact that this swelling does not occur during the postovulation phase of the normal cycle is cited as evidence that the progesterone produced by the corpus luteum is unable to retain in the sexual skin the water deposited there as a result of estrogenic stimulation during the preovulation phase. It was shown experimentally that testosterone propionate and cortin injected into spayed monkeys do not lead to the retention of water that has accumulated in the sexual skin as a result of previous injections of estrone (184). The several questions relating to the histogenesis of the many tissues which are now known to be sensitive to estrogens have been examined in an original way by Zuckerman (186). Cover-slip cultures of rat vagina and uterus treated up to four days with estradiol, though remaining in healthy state, showed no indication of a direct action upon the epithelial cells (33).

From a study of the mechanism of effects of estrogen on the nasal mucosa in atrophic rhinitis, Soskin & Bernheimer (153) considered it most probable that any such effect of the hormone would depend upon its hyperemia-producing and therefore its acetylcholine-producing action. This view was confirmed by the results obtained from spraying the nasal mucosa with a synthetic drug (prostigmin methylsulfate) which is able to reinforce the naturally occurring acetylcholine by inhibiting the choline esterase which destroys it. Another study (51) provided strong evidence that the effects of estrogen may be divided into specific effects which do not depend upon hyperemia and those which are secondary to the

production of hyperemia and, therefore, associated with the acetylcholine-liberating action of estrogen. Astwood (7) found that 2.0 μ g. estradiol given six hours before sampling, was followed by a marked increase of tissue water in the uterus of rats when the injection was made during diestrus and proestrus. During proestrus and estrus this reaction was strongly inhibited. Evidence was obtained that this inhibition is due to an ovarian secretion having the properties of a corpus luteum hormone.

Effects of sex hormones on kidney action are inadequately known and the current contributions are in poor agreement, though species difference and different levels of dosage may account for the dissimilar results. In men, and in women after the menopause or castration, doses of about 600,000 I.U. daily for ten days caused no detectable change in urinary volume (150). In pigeons the second of two massive doses (10 mg.) of dihydroestrone, given at an interval of five days, nearly doubled the water intake (and presumable kidney excretion since there was no gain in body weight) during the four days following the second injection (13). In normal and hypophysectomized rats huge doses (10 mg. daily) of progesterone markedly increased the output of urine (146). This diuresis was much more marked in the hypophysectomized rats, attaining daily 15 to 50 per cent of the body weight. In the operated animals progesterone was further observed to protect the animal from water intoxication.

An acute antinarcotic and antitoxic effect of the estrogenic hormones has been extensively studied and discussed by Stortebecker (159). Castration is said to decrease resistance to alcohol intoxication, while treatment with estrone restores the normal resistance. Estrogens also increase resistance to magnesium and potassium cyanide. Neither vitamin D, progesterone, thyroxine, parathyroid hormone, nor the gonadotrophic factor of the pituitary had any effect on magnesium narcosis in spayed animals. In castrate female guinea pigs or pigs with atrophic ovaries, estrone treatment produced abnormal fat accumulation in the parenchymatous organs, especially the liver, and a tumor-like fat accumulation in the parametrium, consisting of adipose tissue, connective tissue, and phagocyte-like cells. The antinarcotic and antitoxic effects of estrogens are considered probably due to: (a) changes in the nervous system which are connected with calcium metabolism and which may thus influence the permeability of cells, (b) an in-

crease in the general strength and capacity of the organism, and (c) increased detoxification processes in the liver. Sprunt (154) found that estrogens or the pseudopregnant state increased the ability of rabbit skin to neutralize higher proportions of injected particles of vaccine virus. Kochakian (65) observed that estradiol dipropionate in doses of 0.25 and 0.1 mg. was fatal in a particular strain of mice, but was more toxic in females than in males. The addition of testosterone propionate in four times the quantity of estradiol nullified partly but not completely the toxic properties of the estrogen.

Stilbestrol is reported to cause inhibition of smooth muscle and contracture of striped muscle and to have no effect on heart muscle (24). In tests on the influence of diethylstilbestrol on the spontaneous activity of male rats, Hoskins & Small (58) obtained consistent augmentation of activity as recorded on revolving cages. The initial activity was usually restored in from one to two weeks after treatment was discontinued. A similar study (59) of the effect of testosterone propionate on the level of bodily vigor in senile rats disclosed no significant augmentation of muscular activity. In male and female dogs both stilbestrol (in 4 to 10 mg. doses daily) and estradiol benzoate abolished the formation of new erythrocytes, producing an early agranulocytosis followed by leucopenia and thrombocytopenia accompanied by hemorrhage (6). Observations of value have been made on the relationship between the thymus and the sexual organs. Chiodi (22) castrated rats at thirty days and observed that their thymi were heavier than those of their controls during the period between forty-five and two-hundred-fifty days of age. Histologically the glands of the two groups showed no differences at the various ages. The injection of testosterone propionate or estrone caused atrophy of the thymic parenchyma of normal and castrate albino rats of either sex.

Certain studies have supplied further information concerning the actions of sex hormones in evoking and antagonizing production of tumors. The pathological changes produced in normal and spayed rats by prolonged dosage (53 to 146 days) with different androgens and estrogens, alone and in various combinations, were fully reported by Korenchevsky & Hall (66). As observed on simultaneous administration of the two types of hormones, these changes include severe metaplasia of the uterine epithelium, adenoma-like overgrowth of the uterine glands, cystic glandular

hyperplasia in the uterine mucosa, and the formation of cysts, sometimes very large, in the ovaries. These changes were histologically similar to like pathological conditions which occur in women, and they confirm the view that progesterone is able to prevent or diminish the metaplasia into squamous cells of the normal columnar epithelium of the uterus. Lipschütz and associates (81) report results favorable to the theory that the development of uterine fibromyomas in women is due to a disturbance of the normal balance between follicular and luteal hormones and of their normal timing, and that progesterone may prove therapeutically useful against fibromyoma. From other tests (80) made on guinea pigs the tumor-producing action of stilbestrol was found to be much greater than that of estrone or estradiol when equal quantities are compared. Mohs (95) observed that the transplantability and rate of growth of a pure mammary fibroma were enhanced by the presence of physiological levels of estrogens in the host rats. Thus, in females and in estrogen-injected castrates of both sexes the success of transplantation and rate of fibroma growth were greater than in males and noninjected castrates. Physiological levels of androgens had no demonstrable effect on this tumor. The tumor-stimulating effect of estrogen injections was greater in male castrates than in female castrates. The responses of mammary fibroma to hormonal influences resemble those demonstrated for mammary adenofibroma. With the onset of malignancy the connective-tissue tumors lose their responsiveness to hormonal influences.

Effects of sex hormones on ovulation and closely related topics are reviewed in the chapter on reproduction in mammals. The actions of the sex hormones on intersexuality, on plumage change in birds, and on related questions are reserved for the chapter on developmental physiology. The effects of sex hormones on the mammary glands and on reproductive behavior are considered in subsequent sections of this review.

PROLACTIN

The considerable period of active study of this hormone which has not been covered in previous volumes of this *Review* is included in the present statement. It is now obvious that much of the organism—including assimilative, endocrine, and neural mechanisms—becomes involved in the initiation, augmentation, or main-

tenance of lactation. The term "lactogenic hormone" is, therefore, a wholly incorrect name or synonym for that hormone which was found to initiate milk secretion in prepared mammary tissue and which, following its isolation and establishment as an entity, was called prolactin. Lactogenesis involves several hormones and many other things; prolactin is a specific substance which besides stimulating prepared mammary tissue to secretion of milk is now known to bear still other relations to reproduction and to have still other actions. A full recognition of this distinction is both useful and important.

Occurrence and assay.—Prolactin is probably produced in the hypophyses of all vertebrates but the amount momentarily present (or possibly stored) in the anterior pituitary is more plentiful in some species and at certain stages of the life cycle than others. Contrary to the results of an earlier study, a later report (118) states that the pituitaries of fetal calves contain less prolactin than do the glands of adult or pregnant cows, while a higher concentration of the hormone was found in glands from dairy cattle than from beef cattle. In both earlier and later studies a somewhat higher concentration was found in glands from pregnant or lactating cows than in those of heifers. A comparison (20) of the prolactin content of pituitaries of sheep, ox, man, pig, and horse indicated progressively smaller amounts in the order mentioned; only 4 per cent of the quantity recovered from the sheep or ox was obtained from the glands of the horse.

Estrogen administration was observed to increase the prolactin content of the rat pituitary as measured by implantation of the glands from treated and untreated rats locally over the crop-sacs of pigeons (119). Pituitaries of female guinea pigs measured similarly gave lowest values during diestrus and greatest during lactation; during estrus there was more of the hormone than in early pregnancy but less than in late pregnancy (114). Again, Holst & Turner (54) thus found that in both the guinea pig and rabbit the prolactin content of the pituitary fails to increase during early pregnancy, does so only slightly during late pregnancy, but notably increases following parturition. Both species supplied evidence that the absence of nursing results in an increased prolactin content of the pituitary and that the act of nursing quickly leads to its diminution in the pituitary. It was, therefore, concluded that the stimulus of nursing in some way causes a discharge of prolactin

from the pituitary. Small amounts of prolactin have been found repeatedly in human urine *post partum*, and it is alleged (31) that two peaks of excretion occur within the normal cycle, during menstruation and at ovulation. Rabald & Voss (111) report recovery of a prolactin-like substance from livers of healthy beef and hogs, but not from horse liver. Studies made on pituitaries of pigeons and rats provide evidence that prolactin is formed in the eosinophilic cells (143a).

It is advisable to correct an error of statement in last year's *Review* concerning the pituitary hormones for which an international unit was established at the third conference on the standardization of hormones held in Geneva in 1938. Such a unit of prolactin was there established. That conference agreed that the international unit of prolactin should be so defined as to conform as closely as possible to the existing Riddle-Bates unit; that recognizable assays made to determine its value must depend upon measurement or observation of growth produced in the crop-gland of the pigeon or dove, whether as the result of systemic or of local administration; and specifically, "this unit is the activity contained in 0.1 mg. (100 gamma) of the standard preparation" (162). The preparation itself was found to contain 10 units per mg. and was made ready for distribution (from the Department of Biological Standards, National Institute for Medical Research, Hampstead, London) on November 1, 1939.

Useful comparisons of methods of assay are available (14, 128) and some of the conditions affecting these assays have been investigated. The earlier observation of Folley & White (37) that large doses of estrone reduce the response of the pigeon's crop-sac to prolactin led to a study of the mechanism of this action (13) with results which indicate that the diuresis produced by high doses of estrone may cause a more rapid elimination of prolactin by the kidney. Results obtained with the intracutaneous crop-sac micromethod for prolactin assay were also shown (12) to be much affected by the volume of fluid used in the test. When the volume used was 0.05 ml., four times as much prolactin was required to produce minimum stimulation as when the volume was ten times larger (0.5 ml.). There seems to be no simple explanation for this finding that the more dilute solution, i.e., one covering a wider area of crop-sac with the solution, is the more effective. It is clear, however, that assays made by this method are of little quantitative

value unless this physiological factor is regarded and kept constant.

Applications and actions of prolactin.—Estrogenic hormone, though certainly helpful in the preparation of mammary tissue for one true action of prolactin, apparently becomes an agent adverse to milk yield after lactation is established. In the rat the inhibition of lactation was observed after estradiol benzoate, this action being accentuated by simultaneous administration of pregnancy urine preparation (115). Lactation in the rat was also inhibited by stilbestrol (104). In the goat, however, stilbestrol rubbed into udders for thirty days is briefly reported to induce lactation (38). Kawano & Nakano (62) reported that subcutaneous injections of urine from pregnant cattle into virgin, adult, nonpregnant gonadectomized rabbits caused the animals to lactate within seven to thirteen days. The milk produced was colostrum-like at first and later appeared normal. In the human, estrogens have been used to suppress or inhibit lactation in acromegaly (156) and also when the milk flow is excessive (74); testosterone also has been used to stop milk secretion following parturition (68, 13b).

Stewart & Pratt (157), using subcutaneous injection of one thousand units of prolactin daily from the sixth to ninth day *post partum*, inclusive, obtained no significant increase in milk secretion from a group of fourteen patients whose secretion was less than 250 cc. on the fifth day. A similar group of ten mothers served as controls; after leaving the hospital their nursing record was not significantly different from those that received prolactin. Stewart & Pratt conclude that the action of prolactin in animals and women is not analogous. Kenny & King (63) used prolactin in the treatment of forty-three women with deficient lactation, beginning at different stages up to the third month *post partum*; forty-three other women, on whom other galactogogues and routine methods of encouraging lactation were practised, served as controls. In 74 per cent of the treated cases, and in only 21 per cent of the control, lactation became sufficient for the whole need of the baby until weaning at the sixth to seventh month. The complete failures included 19 per cent of the treated and 63 per cent of the control. The total dose of prolactin was nine hundred units, spread over five days in diminishing doses and given intramuscularly twice daily. No local, or systemic, ill effects were observed, and the milk produced was of normal chemistry and quality. It was recommended that treatment of these cases begin early *post partum*.

Other actions of prolactin have been reported from animal experimentation. Splanchnomegaly, exhibited in the liver, pancreas, and intestine of pigeons, is associated with the action of prolactin (13a). This report indicates that body weight and appetite are especially increased by prolactin in pigeons. Large doses of prolactin (probably not entirely free from serum protein) from ox pituitary injected daily into adult rabbits or young female monkeys for eighteen or more weeks resulted in the production of antisera capable of inhibiting the growth response in the crop-sac of the pigeon and also probably reducing milk secretion in lactating mice (179). Preparations of prolactin from ox and sheep glands, apparently free from serum protein, were found to be antigenically indistinguishable; the serum of rabbits injected with hormone from either source usually partly inhibited the action of prolactin on crop-sacs (15).

MAMMOGENIC HORMONES

Numerous old and new studies clearly prove that hormones of the ovary play a part in the growth of the mammary gland; in most species an estrogen induces duct growth (directly or indirectly) and an estrogen plus progesterone assists in lobule-alveolar development. Androgens, too, were later proved capable of replacing estrogens in the induction of growth in the mammary parenchyma. Estrogens have been obtained in small amounts from pituitary and from adrenal tissue. Still more recently desoxycorticosterone acetate and some phenanthrene and stilbene compounds not now known to occur in the body are reported to cause duct development in normal and castrate males of the species studied. In hypophysectomized animals, however, the use of estrogens or androgens to develop mammary tissue has usually, though apparently not always, resulted in failure. Such failures and related facts, together with recovery of potent lipid extracts from anterior pituitary glands which have been recently subjected to ovarian hormones, have led some investigators to believe that, in addition to its various hormones of protein nature, the pituitary gland secretes another alcohol-ether soluble hormone (or hormones) with specific ability to induce mammary growth. A satisfactory review of contributions to this subject therefore requires the consideration of literature bearing on (a) the question whether estrogens, androgens, and available phenanthrenes act directly or

indirectly on mammary tissue, and (b) the intimately related question of the elaboration of a specific mammogenic hormone (or hormones) by the pituitary.

Direct or indirect action of sterols.—Mammary involution might be expected to follow the generally adverse effects of hypophysectomy; it has been reported (165) that the involution of the lobule-alveolar system of such rats is not rapid, but requires at least as long as does its development. Since 1935 most administrations of estrogen to hypophysectomized animals have given negative results relative to mammary growth, but the significance of these failures has been interpreted variously. Selye & Collip (147) in 1936 reaffirmed the view that this action of estrone must be through the pituitary. Mammary growth in hypophysectomized rats was not stimulated by 20 to 100 units estrone for twenty to twenty-five days, according to Gomez & Turner (44). Astwood, Geschickter & Rausch (9) emphasized the great importance of reduced nutrition incident to hypophysectomy in the rat, and also of the estrogen dosage, in studies of the mammary development. They observed mammary regression in normal rats on restricted diets of fourteen days' duration in spite of the fact that 5 μ g. estrone was injected daily. Likewise in hypophysectomized rats the duct system regressed with estrone dosage. However, these observers conclude that such failures provide insufficient evidence that mammary growth is mediated by the pituitary. Nathanson *et al.* (98) confirm the importance of the nutritional factor in maintaining mammary tissue in hypophysectomized rats, but think it probable that estrogen here exerts part of its effect through the pituitary. Other failures of estrogens to support or increase mammary growth in hypophysectomized animals were observed in guinea pigs, mice, rabbits, cats, and ground squirrels (78, 165). Noble (105), confirming earlier work, found that progesterone produced no consistent effect in the mammary glands of adult or immature hypophysectomized rats, and concurred in the view that estrogens and androgens stimulate mammary tissue indirectly through the pituitary. Herold & Effkemann (53), as noted in last year's *Review*, reported finding no effect of estrogen in normal male and castrate female rats after severing the nervous connection between the pituitary and midbrain, and further suggested that estrone acts by way of the midbrain from which impulses reach the pituitary and cause it to secrete a mammary growth factor.

When the pituitary is removed during pregnancy the effects on mammary growth are unexpected and important. In guinea pigs operated at the fortieth day, with mammary glands examined nine to twelve days later, a condition resembling that of normal glands at parturition was found (27). In mice it was observed that, even after digital abortion of fetuses and hypophysectomy on the twelfth day, normal mammary changes occur if the placentae are retained (101). The presence of the ovaries had earlier been found unessential for mammary growth at this period (102). That this influence on normal mammary development may result from the secretion of steroid hormones by the placenta, or quite otherwise, is obvious.

Testosterone and other androgens have again been observed to cause mammary development in normal and ovariectomized rats, though in hypophysectomized rats little or nothing more than nipple growth is obtained (105). In each of three women MacBryde (88) obtained good development in a breast rubbed with estradiol ointment (25,000 I.U. daily for two weeks) while the contralateral breast rubbed only with the ointment base showed much less growth. In male rabbits Lyons & Sako (87) carried out similar tests with threshold and sub-threshold doses of estrone in sesame oil for five weeks. In the three rabbits treated with the lower dose (a total of 30 I.U.) no mammary glands were stimulated; in two of three rabbits receiving the higher dose (a total of 300 I.U.) only the glands on the estrone-treated side were stimulated, while the third more responsive rabbit showed slight mammary stimulation on the oil-treated side, but much stimulation on the estrone-treated side. These studies support the view that estrogenic hormones stimulate the mammary glands directly and do not require mediation by the pituitary.

Butcher (18) made the unpredictable observation that, when underfed female albino rats are adrenalectomized, their mammary glands grow much faster than they do in litter mates with the adrenals intact. The gland of the adrenalectomized animal has many more bud-like projections along the course and at the ends of the ducts and covers a larger area. The growth of the glands is similarly accelerated when animals are both ovariectomized and adrenalectomized; accordingly, this enlargement is not due to increased sexual activity resulting from the adrenalectomy. The author suggests that adrenalectomy perhaps allows more sub-

stances which are necessary for the growth of the mammary glands to pass through their capillary walls. In young unoperated or castrated male mice weighing fifteen to twenty-five grams accelerated development of the mammary glands was accomplished more recently with a wide variety of estrogens, androgens, and with desoxycorticosterone acetate (170). This result with the last-named substance has special importance because (a) it adds the adrenal cortex to the few possible sources of mammogenic stimulation, (b) these cortical products are heat labile (in contrast with many estrogens) and more soluble in fat solvents than estrogens, (c) such products are probably released from the cortex in increased amounts by estrone dosage in normal rats and pigeons but not, or to a less extent, after hypophysectomy (94, 148), (d) desoxycorticosterone (the only adrenal derivative hitherto tested) was observed in the study now under review to have greater mammogenic potency per milligram than either of the five androgenic substances tested, and (e) mice of just the type utilized in this study are also used by Turner's laboratory for assay of the mammogenic activity of the pituitary tissues and extracts to be described in the following section.

Mammogenic potency of pituitary tissues and extracts.—Implants of normal pituitary tissues were followed by no appreciable mammary development in many tests made on rats by Loeb & Kirtz (83). Positive results on both ducts and lobules were secured in other tests on hypophysectomized male castrate and noncastrate guinea pigs when the implanted tissue was from rats previously treated with estrogen (44, 44a). In hypophysectomized male and female guinea pigs a few tests made with cortical hormone ("eschatin") alone or in combination with an estrogen (not named) demonstrated no mammary growth. Moreover, some further tests indicated the ineffectiveness of thyroxin and of other now recognized pituitary hormones for the promotion of mammary growth. In a later study immature ovariectomized rats and spayed rabbits showed complete mammary development following the implantation during twenty-five to thirty days of fresh (or acetone dried) anterior pituitary tissue from pregnant cows (45). In view of these results a new pituitary principle promoting growth in the mammary gland was postulated by Gomez & Turner and called "mammogenic hormone" or mammogen. See the review by Turner (165).

Nelson (99) reported that mammary development in hypo-

physectomized immature female rats, implanted with pituitaries from estrogen-treated rats of either sex, did not exceed or even equal that which follows the implantation of normal rat pituitaries. His failure to confirm the claim for the existence of a specific "mammogenic hormone" was briefly stated. Repeating Nelson's study, Reece & Leonard (116) also found no difference in potency of pituitaries from untreated and estrogen-treated donors, but they note that implanted glands of both types gave evidence of some aid to mammary growth when the treated were compared with their untreated hypophysectomized control. It is evident, however, that some aid to mammary growth might well result from any and all hormones contained in the implanted pituitaries since these would help to relieve one or another of the numerous disabilities resulting from hypophysectomy.

Albino mice were found to respond to pituitary implants and were considered most suitable for assay of mammogen (79). The technique of this assay of the duct-growth factor involves the daily subcutaneous injection of the fresh macerated anterior lobe tissue, or of mammogen-containing extracts, for six days with autopsy on the seventh. The mouse unit is defined as the amount of tissue or extract required per mouse to produce definite signs of duct development in one or more glands of 50 ± 10 per cent of ten or more male albino mice weighing fifteen to twenty-five grams (78, 79). With this technique the mammogen content of pituitary from pregnant cows was found greatest at one hundred fifty days; that of the dairy cow was greater than that of the pregnant beef cow; that of beef heifers with corpora lutea was 40 to 60 per cent greater than that of pregnancy beef cows at the 150-day peak; steer, bull and fetal pituitaries showed appreciable amounts of mammogen (78). The authors suggest that estrogens lead to the production of a duct-growth factor, progesterone to a lobule-proliferating pituitary factor.

Lewis & Turner (77) observed that acetone and ether drying of anterior hypophyseal tissue resulted in a loss of 60 per cent of its mammogen content. Extraction of the tissue with several volumes of hot ether-alcohol resulted in a preparation (the oily residue) containing one unit per 3 to 4 mg. and including practically 100 per cent of the potency of the fresh tissue. Their most recent publication (78) states that their most potent preparation, tested in fourteen mice, gave 79 per cent positive response at a dosage of

0.25 mg. per mouse. The estrogen content of some preparations was measured and considered far too low to have caused the mammary growth observed. The fact that fresh pituitary tissue containing mammogen caused both duct development and lobule hyperplasia, though the lipoid extracts caused only duct development, was regarded as evidence that the lipoid solvent separated a duct-growth factor from a lobule-growth factor.

The several phenomena and facts recently accumulated on this topic merit both attention and further study. Various considerations suggest that the effective lipoid extracts derived from the pituitary should be assayed quite accurately for their content of the several recognized steroid hormones, and that the effectiveness of these preparations be tested also on adrenalectomized-castrate as well as adrenalectomized-hypophysectomized-castrate animals.

NEURAL RELATIONSHIPS

The effect of hypophyseal stalk transection on the gonadotrophic functions of the rabbit's hypophysis was studied by Brooks & Lambert (16) who found that this operation did not interfere with reproductive activities in six of fourteen male rabbits. These males mated and produced normal spermatozoa. Assays for gonadotrophic hormones, both follicle-stimulating and luteinizing, in the anterior lobes of male and female rabbits several weeks or months after stalk transection usually showed normal concentrations. Though this operation invariably prevented coitus-induced ovulation, other abnormalities in gonadotrophic functions of the hypophysis were usually not detectable. Rosen *et al.* (138) observed that in rats the application of local anesthesia to the nasal mucosa was followed by a prolonged luteal phase of the cycle and also that removal of the sphenopalatine ganglion is followed by pseudo-pregnancy. It was shown that the nervous factor was limited to the nonolfactory portion of the nasal innervation since changes in the sexual cycle did not result from removal of the olfactory bulbs. How or whether the hypophysis is involved in these changes is uncertain. Marshall *et al.* (91) showed that picrotoxin (0.9 to 1.1 mg. per kg.) administered intravenously to rabbits in heat may produce follicle growth, follicle hemorrhages or ovulation. Several other drugs with a stimulating action on the central nervous system failed to produce these effects. The authors note that this failure is possibly due to their own failure to find the

optimal conditions for the action of those stimulants but think it far more probable that picrotoxin has a more elective action on the innervation of the anterior lobe of the pituitary than other drugs investigated.

Additional welcome information was obtained concerning the existence of a nervous factor in the maintenance of mammary tissue and lactation. Hooker & Williams (57) studied the influence of irritation of the nipples upon retardation of mammary involution in mice. Removing the young on the fourth day *post partum* was followed by application twice daily of spirits of turpentine to all nipples during seven days. Involution of the mammarys was thus retarded in thirteen of fifteen tests, and in six cases almost no regression occurred. Turpentine applied on the back, or water applied on the nipples, was without effect. Turpentine applied to selected nipples with others remaining untreated was followed by retardation of involution in all the mammary glands. Excision of certain nipples resulted in great diminution of milk production and also in marked retardation of involution in such operated glands when the remaining nipples were suckled for ten to twelve days.

Weichert (172) showed that lactating rats inseminated during the *post partum* estrus had gestation periods ranging from twenty-two to twenty-six days depending on how many young are suckling. A study of the accompanying ovarian conditions, together with their modification through injections of a prolactin followed by small doses of progesterone, led to the conclusion that suckling may stimulate the production of prolactin by the hypophysis with a corresponding inhibition of release of gonadotrophic hormones. The time of release of the ovarian hormones necessary for implantation may thus vary with the strength of the sucking stimulus and be dependent upon the number of suckling young. Labate (69) allowed three control rabbits to become pregnant and on the twenty-fifth day of pregnancy performed Caesarian section and carefully observed the onset and duration of lactation. Two other female rabbits were sympathectomized by removing all the known sympathetic pathways to the uterus, tubes, and ovaries. These operated does were allowed to become pregnant, treated as were other control rabbits, and Caesarian section was performed twenty-seven and thirty-two days after sympathectomy. No difference in the onset and duration of lactation was noted between the two groups, and both groups showed normal reproductive instincts.

The current view that the testicular nerves are either vaso-motor or sensory is challenged by Okkels & Sand (107). According to this study, the nerves of the human testis are far more numerous than hitherto observed, great numbers of the neurofibrils making contact with and ending by touching individual Leydig cells. Martins & Valle (92) report a further study of the endocrine control of the motility of the male accessory genital organs. Longitudinal contractions were recorded in tests *in vitro* of normal and variously treated tissues; the results support the view that in rats testosterone has an inhibitory action on the contractility and excitability of the vasa deferentia, seminal vesicles, and prostates, while estradiol has the opposite effect. Measurements of pupillary diameters in the eyes of cats following injections of morphine, atropine or *d*-pseudo-ephedrine, with subsequent injections of progesterone (30 mg.) or testosterone (40 mg.), led Effkemann (30) to infer that the latter substances have parasympheticotrophic, while estrogens have a sympheticotrophic action.

Estradiol benzoate, in doses of 20 R.U. daily for twenty days, injected into male rats are reported by Uotila (168) to act on the anterior pituitary without the mediation of any modifying effect on the pituitary stalk. The cogent evidence for this conclusion includes the observation that after estrogen administration the anterior pituitary undergoes hypertrophy and shows the same cytological changes whether the stalk be intact or severed; and also that the testicles and seminal vesicles atrophy in both groups. It was observed that the adrenals hypertrophy equally after estrogen treatment in intact and stalk-sectioned animals; this fact was cited as indicating that estrone also affects the corticotrophic function of the anterior hypophysis without any mediation or modification of the pituitary stalk. This inference is of doubtful validity since the reported inability of estrogen to induce cortical hypertrophy in hypophysectomized rats may rest upon secondary effects incident to total pituitary loss, and section of the pituitary stalk may fail to produce those essential secondary effects in the organism.

Dey *et al.* (28) have described the disturbances in reproductive functions of female guinea pigs caused by lesions in the hypothalamus between the optic chiasma and the attachment of the infundibular stalk. These lesions caused sterility and a variety of disturbances in reproductive functions. Clark *et al.* (23) studied

the visual pathways concerned in gonadal stimulation in ferrets, and considered the bearing of their results on the question of pituitary innervation. At the beginning of the nonbreeding season, lesions were made at various levels in the visual pathways of the brains of female ferrets and the animals thereafter were exposed to six and one half hours of bright illumination at the end of each day in order to learn whether heat could be thus induced in anestrus. Ferrets whose optic nerves had been divided either did not come into heat at all, or they came into heat much later than all of the controls. The normal response to visual stimulation did occur, however, in the absence of the anterior corpora quadrigemina, when all retinal impulses to any part of the midbrain were interrupted, and when retinal impulses to the dorsal nucleus of the lateral geniculate body and the visual cortex were completely interrupted. The observers suggest that the visual response depends on impulses passing either to the ventral nucleus of the lateral geniculate body, or to the subthalamus by way of the accessory optic tracts. The inference was made by Rasmussen & Gardner (113) that there are at least 100,000 fibers in the infundibular stem of man and that they are derived almost wholly from the supra-optic nuclei. Forty specialists have participated in an important summary of present knowledge of the hypothalamus (40).

REPRODUCTIVE BEHAVIOR

The several types of behavioral response related to reproduction have been actively studied with results which continue to confirm the view that hormones play important parts in these responses and, in certain cases, these results assist in the identification of the hormones specifically involved in these various types of behavior.

Testosterone propionate has been observed to induce premature copulatory activity in certain immature males and to elicit male copulatory acts in young and mature females. Daily injections of 0.62 mg. into male rats on and after the ages of twenty-two to twenty-six days starts copulatory acts about twenty days prematurely, and the pattern of these copulatory acts was found by Stone (158) to be like that of normal untreated males. In the American chameleon, *Anolis*, pellets of this hormone enlarged ovaries and induced estrus behavior in females. It induced aggressiveness and a male type of courtship and copulation behavior

in immature or adult females, either normal or castrate, and led to full sex activity in immature and adult castrate males (103). For the act of copulation, female guppies (*Lebistes*) take an oblique position, and in water of 28°C the female fish assumes this position at regular intervals of four to six days. In colder water this rhythm is absent. It was observed by Jaski (61) that the males of this species produce an unidentified substance which, with a latent period of one day, causes females to take the oblique position and to do so even in water colder than 28°. Administration of various androgens were observed by Hamilton & Golden (49) to produce definite cock-like behavior in either newly hatched female chicks or in mature hens. The degree of crowing behavior was not proportional to the degree of comb growth but seemed largely dependent upon factors in the individual bird in addition to the degree of androgenic stimulation. In general the response of females to a particular level of androgen dosage was somewhat less than that obtained from males.

Modification of the social order in flocks of hens by the injection of testosterone propionate has been described by Allee, Collias & Lutherman (2). When injections were made into low-ranking individuals in these mixed flocks of treated and untreated White Leghorn hens, a rise in social status was observed in each adult hen thus treated, an injected individual eventually occupying the top position in each flock. In one flock of younger pullets, where peck-order had just become fixed after an extended period of fluctuations, the injections produced no changes in the social position within the flock. Even in this case, however, the hormone-treated pullets were more aggressive and more successful in initial contacts with strange birds than they were prior to androgen treatment. In two flocks of battery-reared young pullets studied while the social order was beginning to be established, the injected birds eventually dominated the flock. Other observed effects of the treatment included larger size of comb, retardation or suppression of egg-laying, initiation of crowing, and a male type of courtship in some cases. These several last-named changes vanished soon after cessation of treatment; but higher social position, once won, was retained. A comparable study of effects of estradiol on social organization in flocks of hens yielded less striking results (1), though in general these were the opposites of changes effected by testosterone. When these same two hormones were simultaneously ad-

ministered to male rats the copulatory ability of the rats was unaffected (96).

Testosterone propionate administered to female canaries was observed to cause suppression of the female reproductive functions and initiation of the following male traits: singing, courtship behavior, peck-dominance over untreated females, and appearance of the male type of anal region. In the presence of receptive females, however, these treated females did not copulate as males (151). Another group of five female canaries which showed no ability to sing prior to treatment was injected intramuscularly with 5 mg. testosterone propionate every three or four days until singing occurred. Four of the five sang the typical male song, one of them beginning after two and others after three injections. The fifth female behaved like a normal male and made attempts to sing. The song of these birds had less volume than that of males and persisted from five to thirteen days after their last injection (75). Still another group of four female roller canaries were isolated in sound-proofed cages and given thirteen daily injections of 2.5 mg. testosterone propionate. These birds began male-like singing on the twelfth to the twentieth days after first treatment (11).

It has been stated that a heavy excess of vitamin B₁ has an untoward effect on lactation and the nursing instinct in rats (108). Rodewald (137) produced estrus in castrate white mice by subcutaneous injection of proper quantities of hematoporphyrin or of protoporphyrin and considered this due to stimulated output of gonadotrophic hormone of the hypophysis or to activation of native estrogenic substance. This observation strengthens the probability that the output of still other pituitary hormones may be increased by other nonhormonal substances. In the initiation of maternal or parental behavior in rats, prolactin seems to be especially involved. Other pituitary hormones, and urinary luteinizing hormone, estrone, progesterone, and cortin were earlier found ineffective or inhibitory to broodiness in the fowl, but Riddle & Lahr (131) briefly report that pituitary (not chorionic) luteinizer, intermedin, progesterone, desoxycorticosterone acetate and phenol are also fairly effective in releasing this behavior in normal rats. This report did not resolve the important question of whether these several partially effective substances do or do not cause a release of prolactin from the rat's own pituitary.

Some information has been obtained on the part played by

hormones in the migration from land to water which occurs in the American newt, *Triturus viridescens*, when it approaches maturity at three to five years. Reinke & Chadwick (121) induced efts in the land phase to adopt a water habitat by intramuscular implants of adult pituitaries derived from *Triturus*. They also found (122) that such implants were effective in efts whose thyroids and gonads had been removed. An attempt by Chadwick (19) to learn whether the pituitary factor responsible for this water-drive is peculiar to *Triturus* or is widely distributed in other vertebrates was partly successful. An extract (Antuitrin G) of mammalian pituitaries known to have growth-promoting power was used in these tests. Large efts ranging from 60 to 85 mm. in length, either normal or gonadectomized, were driven to water within five days by injections of 2 to 3 R.U. of this extract. Similar quantities failed to cause efts of less than 55 mm. in length to enter the water and also failed to affect large thyroidectomized efts. The suggestion that the various results indicate that the water-drive is induced by the growth hormone would be much better supported if the extract used did not contain several anterior pituitary hormones.

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STATION FOR EXPERIMENTAL EVOLUTION
CARNEGIE INSTITUTION OF WASHINGTON
COLD SPRING HARBOR, NEW YORK

REPRODUCTION IN MAMMALS

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Washington, D. C.

With respect to the element of time the organs of reproduction offer to the investigator a challenge probably matched by no other living system. In most systems the predominating reactions occur on one or another characteristic time scale. The velocities may be in milliseconds as in parts of the nervous system, minutes or hours in the gastrointestinal responses to humoral agents, or, in days and weeks as in the endocrine system. The principal reactions in the organs of reproduction are not confined to any one time scale. We are confronted with phenomena which take weeks or months for completion and which involve intermediate steps in a progression from one magnitude of velocity to another. As a result our records in this field of study are episodic. We lack the continuous records necessary for the proper alignment and temporal analysis of the several contemporaneous and related reactions. It is impossible to appreciate the challenge of such a situation without examining in detail some of our current problems.

Central nervous system and reproduction.—The reactions involved in mating behavior are on a time scale familiar to neurophysiologists. In some species, however, the act of mating initiates a chain of reactions in which the neural transmission of the excitatory state to the pituitary in a matter of seconds leads to the discharge of this gland over a period of an hour or more, and this in turn, provokes a series of responses with latent periods of hours or days and with a total time course of weeks. These phenomena are most readily seen in the female cat, ferret, or rabbit, in which ovulation does not occur without the stimulus of coitus or its equivalent. In this respect these species would appear to be set apart from those in which ovulation occurs spontaneously, but in reality the same fundamental physiologic pattern is seen in the rat and the mouse. In these forms it is pseudopregnancy rather than ovulation which is dependent upon this neuroendocrine sequence. This fundamental similarity is emphasized by the ovarian aberration found in a strain of rats with persistent estrus. In these animals, just as in the rabbit, ovulation occurs only after coitus (36).

Coitus is not the only effective stimulus for the response. Earlier studies had shown that mechanical or electrical stimulation of the cervix of rats, and electrical stimulation of the brain stem in rats and rabbits are also effective (17). Recently it has been reported that ovulation in the rabbit can be induced by the intravenous injection of some convulsant drugs (71) and salts of copper or cadmium (34). Section of the pituitary stalk, which prevents postcoital ovulation, also abolishes the ovulatory response to copper salts (17), so that in both cases the stimulus is transmitted to the pituitary over some pathway through the stalk. Likewise, the administration of adequate doses of progesterone to estrous rabbits blocks both the response to coitus (67) and to copper salts (39). The exact site of the progesterone inhibition is not known, but it is either on the pituitary itself or at some point more central in the neuroendocrine pattern (67, 68). It should be noted that the doses of progesterone sufficient to inhibit postcoital ovulation may not suffice to block the copper reaction in all animals (39). Moreover, in pseudopregnant rabbits the natural level of progesterone is high enough to prevent postcoital ovulation but it is not high enough to prevent an occasional response to copper or cadmium salts (34, 39). There have been no reports of attempts to produce pseudopregnancy in the rat by the intravenous injection of copper salts but theoretically this should be possible. It has been demonstrated, however, that the neuroendocrine response in the rat may be inhibited by progesterone, for adequate doses of the latter prevent the pseudopregnancy response to electrical stimulation of the cervix (5).

Obviously the phenomena of pseudopregnancy in these several species reveal the neurohypophyseal connections as components of a functional unit, and, as in other systems, we might expect to find such a unit employed in more than one complex reaction. Yet, there is no satisfactory evidence to support the claims that the integrity of the neurohypophyseal connections is essential for the normal cyclic changes in spontaneously ovulating forms. The only evidence for such claims is from experiments involving pituitary transplantation or stalk section (17) and this evidence is counterbalanced by opposite results in similar experiments. The failure of pituitary transplants to maintain normal ovarian function does not necessarily indicate that normal cyclic pituitary function is dependent upon intact neural connections with the hypo-

thalamus. A transplant, even though functional to some degree, may be restricted to a subnormal level of activity due to circulatory limitations in the unnatural position. In small animals with a short pituitary stalk it is quite possible that the operation of stalk section involves some injury to the pituitary. A recent contribution to this question is the study of Dey *et al.* (27) in which small electrolytic lesions were placed in the hypothalamus of female guinea pigs. In most of the animals it was no longer possible to elicit mating behavior after the operation although twelve of the fifty animals exhibited constant vaginal cornification. Since the body weight of these twelve animals fell below that of the others it is difficult to say whether the persistent vaginal cornification was produced directly by the operation or indirectly through some nutritional derangement. Of the total of seventy-four operated animals about one half displayed regular vaginal cycles and thirteen of these females became pregnant. Properly timed necropsy of the nonpregnant females disclosed fresh corpora lutea, which were evidence that ovulation accompanied the vaginal cycles. Since microscopic examination of the hypothalamus revealed no correlation whatsoever between the limits of the lesions and postoperative ovarian function, nor between the latter and the presence or absence of diabetes insipidus, these experiments offer no support for the thesis of hypothalamic regulation of the estrous cycle. The experiments of Brooks on stalk section in the monkey are as yet incomplete (17), but the recent report of Dandy (25) is of extreme interest. In a young adult woman "complete transection" of the pituitary stalk was followed by normal menstrual cycles which were interrupted only by two pregnancies. In both pregnancies labor and lactation were entirely normal, the only stigma being the continued though mild diabetes insipidus which appeared soon after the operation.

To postulate an independent and inherent rhythm in the pituitary of polyestrous animals is not to deny the existence of some regulation from the nervous system. The predominance of nocturnal estrus in rats, mice and guinea pigs (10, 94, 109) and the twelve hour shift of estrus and ovulation with an experimental shift in the lighting regime (94) strongly suggest some participation of the nervous system. Nevertheless, this participation need not involve any direct effect of light transmitted over the neurohypophyseal connections. The synchronization of the estrous cycle with the light cycle

may be effected by the metabolic alterations which are concomitants of the increased physical activity of the rodent during the night hours. Similarly, the light induced acceleration of sexual maturity in the rat (37, 64), the increased size of sex accessories and the greater sensitivity to gonadotropic extracts (26) could all be either direct effects of light transmitted to the pituitary through the central nervous system, or indirect effects of the increase in general metabolism during accelerated growth (55). The importance of this point is emphasized by the relatively slow growth of the dark room animals listed in the data of one worker (64). Completely unexplained is the mechanism of the production of pseudo-pregnancy in the rat by local anesthesia of the nasal mucosa or by bilateral removal of the sphenopalatine ganglia (86).

Environment and seasonal rhythms.—Regardless of the exact mode of transmission it is likely that light is important among the environmental influences responsible for many of the seasonal changes in the reproductive system. This is especially true in regard to the more subtle seasonal changes in our common laboratory animals kept under relatively constant conditions of temperature and diet. We may recall, for example, the report of seasonal variation in gonad size and gonadotropic sensitivity in the laboratory rat (26, 44). In the rat also there is some evidence of an orderly variation in the age of sexual maturity on a time scale and pattern suggestive of a true seasonal variation; i.e., most rapid maturation in the early spring (41). Despite the widespread belief to the contrary, the laboratory rabbit on a constant dietary regime shows a marked seasonal variation in the incidence of estrus, varying from 80 per cent in the spring to about 25 per cent in the autumn (38). Paralleling these changes is a seasonal variation in the gonadotropic content of the rabbit pituitary (42). It should be noted, however, that there is no correlation between the hormone content in the pituitary and the functional activity of the reproductive organs; a low hormone level being found in the pituitaries of winter animals fully in heat, and high levels in spring animals whether they are in heat or not (42, 43).

In some species the sex cycles continue throughout the year but fertility is subject to seasonal variations. Such is the case in zebu cattle (2) and in the monkey. In the latter animal the summer sterility is known to be due to the failure of ovulation during the summer months. The possibility of seasonal changes in the fertility

of the rat has not received much attention. There is a rather general impression that the group fertility in rat colonies is lowest in the autumn months but this impression has not yet been verified by an adequate analysis. Of course, the crude fertility rate in rabbit colonies would be low in the autumn due to the low percentage of females in heat. Yet those animals which do mate have just as high a fertility rate as animals mated in the spring. The only detectable difference is in the number of corpora lutea formed after each mating. In 150 matings about equally divided between spring and fall the average number of corpora lutea per animal was 11.23 and 9.59, respectively. This difference is small but it is over four times the standard error of the difference between the means and is therefore significant (40).

In addition to these annual rhythms in gonadal function there are others of undisclosed origin which follow almost identical patterns. The curve expressing the seasonal changes in the blood calcium of pregnant women (14) could almost be superimposed upon the curve describing the hormonal variations in the rabbit pituitary, i.e., with lowest values in January and the peak in early spring. This, of course, may be pure coincidence. A very similar curve is followed by the toxemias of pregnancy. In this country and in Europe (15, 31), this complication rises to its highest incidence in the spring and reaches its nadir in the autumn. In Hungary the number of cases of eclampsia per hundred deliveries in May was three times the number in November (31). Of more immediate interest to us is the claim for a seasonal variation in the response of castrate mice to a constant dose of estrin at regular monthly intervals, the response in May being three times as great as in November (30). Emmens also found significant variations in sensitivity from time to time but could not discover a definite seasonal pattern (33). His failure to confirm Duszynska may be due to the differences in the methods employed. The individual points on Emmen's curve were calculated from the responses of different groups of animals to varying dosages of estrin. As shown by Emmen's own data the sensitivity in any group of animals is much lower at the first and second injections than in later assays. One may see from the tables in this report that of the ten assays fixing the point for sensitivity in April and May of 1939, seven were obtained from groups of mice injected only once previously—i.e., relatively insensitive animals. We may also note that there are no data for

the following spring and that the points for the intervening winter months were obtained from assays on mice used several times previously. It is possible, therefore, that a true seasonal variation may have been masked. Emmens did find that constant light decreased the response to estrone, but the direction of this effect is not of the kind to harmonize the two sets of data.

The disagreement between the data of Emmens and of Duszynska may very well serve to illustrate the complexities with which time and the environment confront the investigator. Obviously, these recorded variations in the response to estrin are not necessarily variations in the sensitivity of the vagina. They are variations in the reaction of the entire animal, a reaction subject to any change in circulation or metabolism which could alter the rate of absorption, distribution, or destruction of the injected material. We have already cited seasonal changes in human pregnancy which reflect alterations in metabolism and circulation. The hereditary tail necrosis in the rat seems to present another likely example (108). This trait, which appears in the new born before weaning, is transmitted as a recessive character. All grades of the affliction may be seen from a slight hyperemia of the tail to successive amputations of segments at the annular constrictions which appear in the hyperemic member (41). The marked seasonal variation in the incidence (highest in winter, almost nil in the spring) of this condition reveals the interplay between hereditary and environmental factors. To demonstrate the presence of this trait one would have to look not only at the proper time, but at the proper time in a suitable strain of animals. Hereditary tail necrosis is not the only strain difference to be found in rats and it need not be the only seasonal variation exhibited by one strain and not by others. There are recent reports of strain differences in the rate of attainment of sexual maturity (13, 96), growth, and carbohydrate metabolism (48), but as yet no analyses of the interaction between environmental factors and these strain differences. Finally, to add to the confusion in these long experiments, unappreciated trivia of laboratory routine, not described in publications, may become decisive factors in the sum total of environmental influences. An astonishing instance of this kind is seen in the effect of the number of animals per cage upon the age of sexual maturity, an effect demonstrable under conditions which exclude the more obvious factors of overcrowding and competition for food and water (41).

Briefly, we are not sufficiently aware of the controls necessary for experiments on such an extended time scale. We must, therefore, make some alteration in our scientific philosophy. Among the tacit assumptions in almost every scientific article is the universal validity of our results. We have assumed that the results obtained in one laboratory could be repeated at any future date in any other laboratory if only the skeleton of our experimental procedures were faithfully followed. Obviously, such assumption is no longer tenable. To our skeleton of experimental conditions we must add suitable recognition of heredity and include a new dimension—time. Even with such prescriptions negative results in one country may not constitute a refutation of positive results in another country. There remains the variable of place, and this will confront us until we can discern the significant environmental factors and duplicate at will the artificial environment appropriate for our purpose.

Diet and reproduction.—Diet is properly a part of the environment which in nature changes in time and place, but it is less likely to be an unappreciated variable in the laboratory. Yet, in diets compounded of natural foodstuffs an assumption of constancy may be an error. The transmission of the gonadotropic effects of light indirectly through actinically activated food (77) has not been confirmed (59) but variations in known and unknown accessory factors may occur, e.g., the seasonal changes in vitamin A and grass-juice factor in milk. Fortunately, the reproductive system is not much affected by minor dietary changes, but it is profoundly affected by major changes.

We are again reminded that undernutrition severe enough to cause marked weight loss disrupts the estrous cycle of rats (104) and guinea pigs (95). The anestrus produced by boric or benzoic acid (79) may also be nutritional in origin. The continued gain in weight of the experimental animals might speak against such explanation, but it is significant that the acids were effective only when fed, and not when administered parenterally. The previously reported anestrus in rats on a diet with gliadin as the only protein has been confirmed (60). In these experiments of Lafon & Veillet, however, it is stated that the anestrus is not produced by complete ovarian failure. "Ripe follicles and corpora lutea" were found in the ovaries during the anestrus. In such anestrus females the injection of gonadotropic extracts led to a prompt ovarian reaction but

a feeble vaginal reaction. This, plus the apparent resistance of such animals to injections of estrin, led these workers to believe that the anestrus of lysine deficiency was not primarily ovarian. It is to be regretted that the corpora lutea found in the ovaries had not been marked with some vital dye, or dated by some other reliable method, to rule out pre-existing structures.

There are many who would agree that dietary anestrus is not primarily an ovarian failure. They would disagree, however, with the thesis that the anestrus is the result of something other than ovarian failure. After long periods of malnutrition some workers describe the ovaries as resembling those in hypophysectomized animals (56, 95). Because such atrophic ovaries can be stimulated by gonadotropic extracts several investigators have suggested that dietary anestrus is really due to pituitary dysfunction. In support of this hypothesis, Werner cites the failure of the cytological response to castration and the low hormone content in the pituitaries of the undernourished animals. It is difficult to assess the value of the histological findings as criteria for the functional activity of the organ. Perhaps it is pertinent to note that not all normally fed rats react alike to castration, the cytological changes being more pronounced in those individuals which become obese following the operation (88). Hence the cytological response of three of Werner's eleven castrates, despite the marked loss of weight, may be of more significance than the lack of response in the other eight. No more convincing are the data obtained from the gonadotropic assays. Overlooking the limitations of the method of assay, and accepting the figures of Werner as showing actually the relative amounts of gonadotropic substance, the data do not give clear evidence of pituitary failure. To be sure, the hormone content reported for the pituitaries of starved adult females was somewhat lower than for the pituitaries of fully fed controls, but the gonadotropic activity of glands from starved immature females was not significantly different from those of normal mature or immature females. If we were to accept this type of evidence we should be obliged to conclude that undernutrition produces pituitary failure in the adult, but not in the immature animal. Moreover, the gonadotropic activity recorded for the glands of starved adult castrates was much higher than that of starved animals with ovaries intact. On this basis one would have to infer that castration in the adult animal led to an increase in gonadotropic hormone level despite the severe

undernutrition, or that the decrease in hormone level from the original normal value was partially prevented by castration. The absence of control figures for the starved animals at the time of operation prevents any decision between these alternatives. Completely at variance with the data of Werner are those of Hundhausen (56). This investigator finds that undernutrition sufficient to cause a weight loss of over 30 per cent does not decrease the gonadotropic content of the pituitary. Vitamin-B deficiency, on the other hand, does do so.

Actually these assays of rat pituitaries merely indicate a level of hormone at which equilibrium had been established at the time of autopsy. They give no information either about the rate of gonadotropic hormone formation or its secretion into the blood stream. An attempt to get such information was recently made in some experiments (43) on the rapid changes in the rabbit pituitary during pseudopregnancy. In these studies it was seen that rabbits on a completely protein-free diet were able to release to the ovaries the amounts of hormone required for the growth of follicles and the normal corpora lutea of pseudopregnancy. Pituitary assays showed that over and above these amounts the animals were able to build up and accumulate an excess of hormone at a rate indistinguishable from the rate of accumulation in fully fed controls. Significantly, the accumulation of this excess took place at a time when the only protein nitrogen available was that coming from the tissues. If these data may be accepted as showing the rate of formation of gonadotropic hormone, then this process in the rabbit pituitary is not dependent upon the dietary supply of lysine or any other protein nitrogen. Yet, we can accept these data only after some qualification. If the future reveals no other shortcomings, the validity of these experiments rests upon the assumptions made, in lieu of direct measurement, for the estimation of pituitary hormone formation. These assumptions were: (a) the rate of hormone secretion into the blood stream is governed chiefly by the functional state of the gonads, variations in other organs being relatively unimportant; (b) the rate of secretion does not differ significantly between groups of animals with ovaries in precisely the same physiological condition. These assumptions appear to be logical ones, but they may not prove to be correct. For the present it may be said that these studies on the rabbit pituitary do not support the thesis of primary pituitary failure in protein deficiency.

At first thought some species difference might be advanced to explain the disagreement. But even in the rat the evidence is not all in one direction. To the discrepancies already cited we may add the maintenance of spermatogenesis and fertility in male rats (60) on lysine deficient diets producing anestrus in females, and normal testicular structure and sex activity in the inanition of extreme vitamin-B deficiency (35). One might also cite the older experiments of Jackson on very immature rats. With dietary restriction so severe as to permit no gain over the birth weight, the testes weight increased 400 per cent at the expense of other viscera (58). The work of Addis & Lew (1) on more mature rats suggests a similar response to dietary restriction. These workers report that even on a 1 per cent protein diet compensatory ovarian hypertrophy follows unilateral castration. Unfortunately this conclusion is drawn from comparisons of ovarian weights of operated and unoperated animals after equal periods of dietary restriction, with no figures for the actual organ weights at the time of operation. It is, therefore, impossible to say whether any real growth of ovarian tissue occurred postoperatively, or merely a less rapid ovarian weight loss in the animals with but one ovary.

There are two variables, common to all of these experiments, which might contribute to the divergence in results. They are the severity of the dietary restriction and the stage of the deficiency subjected to analysis. The importance of such variables is seen to best advantage in studies on hemoglobin and plasma protein formation. After sufficient time on a basal ration of low protein content, plasma protein and hemoglobin formation become stabilized at basal levels not further depressed by indefinite continuation of the experiment. Substitution of a protein-free diet, or complete starvation, does not influence the rate of plasma protein formation (66, 73), but sharply increases the rate of hemoglobin formation (24, 47). If the process of gonadotropic hormone formation reacted to dietary restriction like hemoglobin, rather than plasma protein, it might be very difficult to locate the precise degree of dietary restriction necessary to depress pituitary function. It is entirely possible that other parts of the reproductive system will deviate from a simple linear progression in their reaction to dietary restriction. Observations limited to the terminal stages of the deficiency might not disclose the mechanism. It seems reasonable to expect that the primary cause of dietary anestrus becomes operative before the

first missed period and should be disclosed by a more searching analysis of the deficiency at this time or very early in the anestrus.

Human reproduction—indirect criteria of ovulation.—The difficulties encountered in the common laboratory animals are greatly magnified when similar problems are studied in the human. Here the time scale extends to such limits that the adult life span of the investigator does not exceed that of the experimental material. It is interesting to consider the design of the experiments necessary to test in the human the applicability of the results of Hoelzel, Da-Costa & Carlson on rats (53). These workers noted a relation between the sex ratio of offspring and the nutrition of the parents, the sex of the better nourished parent predominating. In the three series of experiments, it would appear that the observed deviation of sex ratio was significant in only one (145:100), but the hypothesis receives support from the fact that the direction of the shift in each of the three series was in line with the argument. Still more work is needed to clarify these results although the investigators observed several generations of rats over a two-year period. The equivalent experiment in the human species would be a formidable enterprise.

Less formidable but still beyond our grasp are adequate records of the phenomena of the human menstrual cycle. The precise time of ovulation in any individual woman cannot be predicted, and probably cannot be recorded by any means now at our command. For a proper appreciation of the task one has only to refer to the effort required for the precise orientation of the events in the relatively short cycles of rodents (10, 94, 110)—a task requiring continuous day and night observations of a whole corps of co-operating investigators. Even if adequate criteria were available a comparable orientation in the human would entail heroic efforts. But the criteria employed in the study of the human cycle are open to question, to say the least. The cyclic variations in the rate of reduction of oxyhemoglobin *in vivo* (method of Samuels) apparently do not exist; the individual observations of the proponents being in all likelihood more the result of a fantasy in dim light than any objective measurement (57, 75). Equally remote from any immediate connection with the organs of reproduction is the Takata reaction, essentially a flocculation reaction carried out on systemic blood serum. Ebergenyi, finding a fall in titer about the sixteenth day of the cycle in ten of fourteen women, seems willing to accept this criterion as a sign of ovulation (32). The presence of an equally

great fall during menstruation in these women, and the absence of any fall intermenstrually in four of the women provided no obstacle to this deduction.

In a somewhat different category are other suggested criteria. From a study of the cyclic variations of vaginal pH in eighteen cycles of six women the intermenstrual rise in pH is advanced as a sign of ovulation (112). Continued studies of cyclic variation in basal body temperature are offered for the same purpose (87, 102, 111). In one study the temperature variations were correlated with the appearance of intermenstrual signs "which might be indicative of ovulation" (111). These signs varied from a slight vaginal discharge, perhaps with some bleeding, to a temporary diarrhea, or headache and sleeplessness. In 80 per cent of the cycles in sixty-seven women there occurred a mid-period fall assumed to denote ovulation. In another study the temperature curve was correlated with daily vaginal smears (87). The absence of the expected changes in about one half of the cycles was interpreted as indicating the absence of ovulation, i.e., menstruation without ovulation. Vollman relied on the *Mittelschmerz* as an indicator (102) but made simultaneous observations on basal temperature. In a woman with pronounced intermenstrual pain the temperature change differed from the onset of pain by not more than two days in most instances, though the seventeen cycles studied varied from twenty-five to thirty-three days. It is noteworthy that this difference was always in one direction, pain preceding the temperature change. In another patient with a forty-three-day cycle, pain appeared on the thirty-first day followed some forty-eight hours later by the rise in temperature.

Recently Barton has summarized the results of studies on the bioelectric potentials in 152 cycles of thirty-six women (7). The peaks of potential, which have been accepted as evidence of ovulation, were most frequent in the mid-period, but were not confined to this interval. No part of the cycle was altogether free of potential peaks. In about one fifth of the cycles no significant peaks were found. In others more than one peak was seen during the cycle.

Workers are not unanimous in their opinions of these various criteria. Without citing detailed evidence, Rock and collaborators voice the opinion (84) that the value of the *Mittelschmerz* is highly overrated. Rather inconsistently, Vollman (102) believes he has produced the *Mittelschmerz* by estrin injections and regards tubal

spasm as the likely source of interval pain. From an unpublished study of vaginal smears in six hundred cases Rock *et al.* (84) are convinced that ovulation cannot be detected in this manner. Appropriately, a recent publication is a reminder that follicular vaginal smears are seen in about one half of women after the menopause (8). Also the variations in electric potential and vaginal pH can be reproduced in castrate animals by variations in estrin levels (29, 85). Less criticism has been levelled against the cyclic variations in basal temperature, but it is yet to be demonstrated that the great variety of extragenital factors known to influence rectal temperature are so subordinated in the human female that a pattern imposed by the ovary emerges unmodulated. In the experiments of Rubenstein (87) the absence of the temperature signal in about one half of the cycles may be interpreted in more than one way.

Actually, none of the criteria suggested has been shown to be so inextricably associated with ovulation that one cannot occur without the other. The only way in which we can ever establish an indirect signal of ovulation is by an adequate check against unequivocal observations on the time of ovulation, not only in a large number of women with normal and regular bleeding cycles, but in an equally large number of women in whom there is some temporal dissociation of ovulation and some of the other concomitants of the cycle. Correlation of one proposed criterion with another proposed criterion does not constitute such a check. One commendable attempt at an evaluation of an indirect signal (bioelectric potential) by direct inspection of the ovaries revealed damaging discrepancies even in a small series of cases (84). Some degree of error is unavoidable even when ovulation time is determined by direct ovarian inspection. Unless observations are made shortly after ovulation an accurate estimate of the age of the corpora lutea may be difficult or impossible (21, 22, 50).

So also must we make some reservation in the interpretation of endometrial biopsies and pregnandiol analyses. Perhaps in a great majority of normal women the endometrial biopsy will faithfully register the rise and fall of the lutein phase, but the statistical reliability may not be the same in just those menstrual irregularities in which information is most acutely needed. At least some puzzling surprises may be uncovered as in the instances of secretory endometrium in biopsies taken during the first half of the cycle

(106). The good general agreement between endometrial biopsies and pregnandiol determinations in the sixty-five cases reported by Buxton (19) was to be expected. In view of the technical difficulties inherent in the detection of the small amounts of pregnandiol found in the urine of nonpregnant women we should be prepared to discount some of the discrepancies which will be found. Yet, the finding of normal amounts of urinary pregnandiol (80) in women in whom the uterus fails to register the presence of a functional corpus luteum—such discrepancy is of the greatest interest. It is essential that we appraise pregnandiol for what it is, i.e., a product of steroid metabolism one source of which is the corpus luteum in the human. This, however, is not the only possible source. It is excreted during pregnancy in castrate women (18, 89). It is found in the urine of bulls (69) and may result from the metabolism of the adrenal steroids in man (23). We do not know what part of the total lutein secretion in woman is represented by urinary pregnandiol, for it may not be the only end product and the urine may not be the only path of elimination. Certainly in primates and in the common laboratory animals we must consider some other course for progesterone metabolism since pregnandiol is not found in the urine either during pregnancy or following the injection of large amounts of progesterone (70, 105). Hence several possible alterations in progesterone metabolism, as well as any changes in progesterone secretion, may alter the urinary elimination of pregnandiol. Even if we assume that such alterations of metabolism will be so rarely encountered in woman as to be of more theoretical than practical importance, pregnandiol could be accepted only as a signal of functional lutein tissue, not necessarily of ovulation. Although the development of functional lutein tissue ordinarily is a consequence of ovulation, it may be present in corpus luteum cysts and granulosa cell tumors and be accompanied by secretory changes and a decidual reaction in the endometrium (98). Finally, we must consider the possibility of the secretion of progesterone before ovulation. In at least one species luteinization precedes ovulation (99).

Ovulation time and fertility.—The importance of the element of time is dramatically emphasized by recent studies in fertilization. In general, nature has provided that a swarm of sperm shall wait in the uterine tubes for the arrival of the ova. In polyestrous animals the ovarian cycle and the behavior pattern are so integrated that

the female is sexually receptive for a very limited period just preceding ovulation. A thrifty modification of this arrangement is seen in those forms in which copulation is a prerequisite for ovulation. A slight dislocation of the natural sequence as in the split estrus of guinea pigs (109, 110) and mares (78) may interfere with fertility despite normal gonadal function. In a series of experiments on timed artificial inseminations (11, 12) the success of the operation diminished progressively as the interval increased between the onset of heat in the female and injection of the sperm. In the guinea pig insemination during heat and eighteen, twenty-four, thirty-six, and forty-two hours afterwards resulted in impregnation in 83, 67, 56, 31, 7, and 0 per cent, respectively. The number of abnormal embryos increased as fertilization was delayed so that in the few pregnancies of the thirty-six-hour group there were no normal embryos and no litters were cast. Similar results were obtained in the rat. In no case was normal development seen when insemination occurred more than ten hours after the estimated time of ovulation.

If comparable time relations also prevail in the human where sex behavior is not so fruitfully guided by ovarian influences we can understand the frequency of sterility in marriages between apparently normal men and women. We can but express grateful surprise at the degree of success so far reported for artificial insemination in woman. In women complaining of sterility, Cary reports several successes with single inseminations in the period between the eleventh and fourteenth days of the cycle. In one interesting case with a twenty-four to twenty-six-day cycle, success was obtained with a single insemination on the eighth day after two successive failures in preceding cycles with insemination on the eleventh day (20). Much less comfort for our prevailing views on ovulation time is found in the results of Seymour (90). Insemination on days two, four, and five, of the cycle in three women were followed by pregnancies after earlier failures with mid-period insemination in each of them. In a reply to a request for further information on this point, Dr. Seymour (91) has cited additional cases from unpublished records, a total of twenty-four cases in which pregnancies were obtained following artificial insemination during the days of bleeding. These successes also were secured after repeated failures in the same women with inseminations between the eighth and twentieth days of the cycle. Conse-

quently, Seymour is convinced that in some women ovulation must occur during menstruation rather than in mid-period. This conclusion is not the only possible one, but the alternatives either do equal violence to our notions of sperm survival or raise some questions relative to the reliability of the testimony of the women in these experiments.

The problems raised by these experiments in human reproduction are not limited to the physiology of ovulation. In several cases in both series sterility was encountered despite the demonstrable fertility of the female and with no apparent abnormality in the male. An unusual demonstration is afforded by the series of Seymour. After the apparently normal sperm of one male had failed in the artificial insemination of thirteen women, each of these women became pregnant upon insemination with sperm from another donor. (Incidentally, eleven of the thirteen children were males.) It is to be regretted that our cytological criteria for sperm normality are not more satisfactory, but a recent publication (46) seems to offer great promise. By dark field examination of suitably prepared samples, it is possible to identify a vesicle in the anterior portion of the sperm membrane which becomes visible under these conditions. The percentage of sperm with vesicles is reported to decrease with the age of the specimen, and in fresh samples from successive ejaculates. More significantly there was a high degree of correlation between the percentage of vesicles and glycogenolysis, since the latter had been correlated with the duration of motility. Perhaps the recent studies on sperm metabolism are but tokens of an increasing interest in this subject. Apparently the rate of oxygen consumption in undiluted semen is a good index of the viability of the specimen, not only with respect to the duration of motility but also with respect to fertilization capacity (103). In Ringer solution, however, oxygen consumption is stated to be very low and sporadic (65). Equally contrasting are the effects of external agents in diluted and undiluted semen. Sperm in undiluted semen are stimulated by oxygen and depressed by carbon dioxide (92), whereas in Ringer solution motility is impaired by either oxygen or air (65). Because of the lack of detailed information in either report as to the limits of gas tension under which each effect may be observed it is not possible to weigh the significance of these differences. When such information becomes available it will be profitable to compare these data with the gas ten-

sions prevailing in the tissues and secretions of the female genital tract. The cyclic variations in the pH of cervical mucus (62) may be indicative of changes in gas tension not only in cervical secretions but in the rest of the tract as well. The cyclic penetrability of cervical mucus to spermatozoa, and the cyclic changes in sperm survival, rising and falling with the pH of cervical secretion (62), show a significant peak in the middle of the cycle. There is, however, another peak during menstruation.

Physiology of the uterus; uterine reflexes.—For a recapitulation of the whole gamut of reactions encountered in the reproductive tract we could do no better than to survey the recent studies on the uterus. The fact that little attention has been paid to uterine reflexes does not detract from such survey. Other reproductive organs have shared this treatment. Indirectly, we may infer that uterine reflex responses to the act of mating are not indispensable for sperm transport. The artificial insemination experiments in the rat, guinea pig, and human permit no other interpretation unless such reflexes are initiated by the very mild stimulation incident to the introduction of the sperm. Reflex uterine responses to such mild vaginal stimulation have not yet been reported. Certainly stimulation of this intensity does not lead to the usual postcoital response in the rat (pseudopregnancy) or rabbit (ovulation). Moreover, the work of Genell (45) would indicate that active participation of the cervix is not essential for sperm transport. Immobilization of this portion by an indwelling cannula did not prevent the ascent of sperm. The only study directly concerned with uterine reflexes is that of Robertson (83), who reports that uterine contractions in women were observed in response to emotional stimuli (anxiety over the pain of an impending injection).

Cyclic uterine growth.—Perhaps uterine reflexes are of less functional importance than the very much slower reactions to endocrine stimuli. Of first importance, however, is a type of reaction intermediate in velocity which occurs in the first few hours after estrogenic stimulation. Probably the first uterine response to estrin is hyperemia. A recent estimate of the latent period is two to three hours (54) which differs considerably from the earlier estimates of a few minutes (81). These observed latencies, of course, relate to the response of the animal and not necessarily the uterus. It is therefore somewhat more likely that part of the difference is due to the use of different experimental animals (the

rat instead of the rabbit). On the other hand, this may be another evidence of our need for continuous registration by adequate physiologic techniques in place of the discontinuous, subjective estimates. Even so, the longer estimate of the latency would pair this reaction with the very early increase of tissue water and electrolytes which precedes the more obvious signs of estrin stimulation. Because the estrin hyperemia was not prevented by the systemic administration of atropine, Holden concludes that acetylcholine is not the mediator of the reaction in the rat. As supportive evidence he cites the failure of acetylcholine to induce visible hyperemia even when applied locally after physostigmine. Contrarily, Hechter *et al.* report that estrin hyperemia in the mouse is inhibited by atropine (52). Without accepting the conclusions of either of these papers it might be well to note first that the observations of Hechter *et al.* do not apply directly to the early hyperemia in the uterus since their records refer chiefly to the changes at twenty-four or more hours after the injection. Secondly, we may recall that even in susceptible animals the threshold for full atropinization varies greatly from one organ to another, the vasomotor phenomena being more difficult to block than many others (e.g., in the salivary gland, the vascular response as compared with the secretory). Since rats and mice are highly resistant to atropine, negative results such as Holden's are not decisive, particularly since no evidence is presented that the doses of atropine employed were effective at other sites. On the other hand, the doses employed by Hechter *et al.* were in the lethal range so that the atropine effect may have been something other than a specific-inhibition. In some experiments on the early increase in uterine tissue water, Astwood found that marked dehydration (by injection of hypertonic glucose) inhibited the response, but that atropine was without effect until toxic doses were reached (4). It probably would be fair to say that the recent experiments involving the use of atropine do not support the thesis of acetylcholine as a mediator of the early uterine reaction to estrin, but neither do they conclusively eliminate acetylcholine as a participant.

The difference in chemical composition of the uterus at six and at thirty hours after estrin injection is intriguing. The weight increase during the first six hours is chiefly due to an influx of a fluid relatively rich in sodium and chloride, and relatively poor in potassium, magnesium, and phosphorus (97). Although these latter

elements show some absolute increase together with the absolute increase in total solids, their greatest increase takes place between six and thirty hours, at the end of which time the maximum uterine weight has been reached and the original water and electrolyte pattern has been restored. The extent to which the early weight increase represents an ordinary transudation of a plasma ultrafiltrate, or, a specific component of early growth is problematical. The weight gain in the process of orderly somatic growth is to a large extent a gain of water and electrolytes, but this is accompanied by a characteristic gain in protein (63). No protein analyses of these reactions in the rat uterus are available. The only relevant protein data are contained in the paper of van Dyke & Chen (100). The change of the monkey uterus from an organ of relatively high water, ash, and protein content in the follicular (estrin) phase to an organ of higher solid, and fat content in the lutein phase suggests the transformation of a growing, immature tissue into a more mature one. These data, however, furnish no information about the very early reaction to estrin.

In the intact animal individual variation in the timing of the cyclic ovarian stimulus would make it almost impossible to distinguish the two phases of the uterine reaction to estrin. We have neither a reliable sign of the release of the estrogenic stimulus nor a means of continuous registration adequate to detect the very early uterine response (i.e., during the first six hours after the release of the stimulus). The available data do reveal the time relation between the peak of the secondary uterine weight increase and the vaginal smear (3). We may recall that in the intact animal the estrogenic stimulus is released by the ovaries some thirty-six to forty-eight hours before vaginal cornification (16). In the normal cyclic rat the peak in uterine weight is reached some eighteen to twenty-four hours before vaginal estrus. Hence the estimate of the reaction time of the uterus *in vivo* agrees quite well with the estimate based on the response to estrin injections (about thirty hours). If the uterine and vaginal reactions were simply responses to a single common stimulus it would be possible to cite them as reactions of different velocities showing the temporal dissociation of sexual phenomena arising from a common cause. Astwood is inclined to doubt that the cyclic weight changes in the rat uterus are attributable solely to estrin. He was able (3) to superimpose on these changes an added weight increase in the six hours after an

estrin injection only if the injection were made during met- or diestrus; not during proestrus or early estrus. Since progesterone is capable of inhibiting the early response to estrin, Astwood concludes that the ineffectiveness of added estrin during proestrus and estrus is due to the secretion of progesterone by the ovarian follicles during their preovulatory swelling. Certainly, we must give serious consideration to this possibility, but Astwood's control observations do not altogether exclude a refractory stage in the course of the estrin reaction. The response of the immature rat uterus to a second dose of estrin forty-two hours after a first injection merely indicates that a refractory state of such duration does not follow the excitation produced by 0.1 gamma of estradiol. This degree of excitation may not compare at all with the excitation during the normal cycle and the duration of a relative refractory period could differ under the two circumstances.

Relation of uterine tension to motility, pain, and onset of labor.
—Recent papers (72, 83, 107) on the motility of the human uterus add to the list which apparently stands in conflict with the Knauss thesis of an inactive and insensitive uterus during the lutein phase. In none of these recent studies, however, has the original technique of Knauss been duplicated, i.e., intra-uterine volume not exceeding 4 cc. and recording pressure not exceeding 20 mm. of mercury. The technique of Knauss is not the only one by which valuable information can be obtained. At times there may be some special advantage to recording at higher pressures and with greater distention. The records in these recent studies generally show higher recording pressures in the first half of the cycle than in the second half. The contraction peaks during the first half attained much greater tensions than in the lutein phase, often exceeding 100 mm. of mercury. This probably arises from the fact that under the influence of the corpus luteum the uterus becomes more tolerant to internal distention. It is quite clear that during the lutein phase the human uterus will exhibit spontaneous motility and will respond to pituitrin if it is sufficiently distended. But one cannot define the spontaneous motility of the uterus and its pituitrin sensitivity except by reference to specific conditions of intra-uterine volume and tension. Cyclic determinations with constant volume and variable tension may yield results entirely different from determinations with constant tension and variable volume.

If the uterus resembled other hollow viscera, we should expect

some relation between uterine tension and pain. If such relation does exist the records of Wilson & Kurzrok (107) indicate that it is not a simple one. Records taken during periods of uterine cramp revealed contractions with peaks of tension not exceeding those seen at other times without pain. Furthermore, the cyclic pattern of motility in women with dysmenorrhea did not differ noticeably from the normal. Such records led the authors to conclude that dysmenorrhea is related to an altered perception of essentially normal uterine contractions. A similar conclusion was reached by Moir (74). Although he noted a correlation of labor pains and the uterine contractions, he observed equally vigorous contractions some weeks before and shortly after labor without pain. Moir also made some observations on the uterine sensitivity to a variety of stimuli, such as cutting, electrical stimulation of the mucosa, pressure, etc. The results are all reminiscent of earlier work on other hollow viscera. Pain was not produced by any stimulus except distention. Perhaps the most interesting parallel with the production of pain in other viscera is the time lag between the contraction peaks during labor and the sensation of pain. Just as in the case of hunger contractions, pain is apt to be felt as the tension is falling rather than during the tension rise. Another parallel with the physiology of the gastrointestinal tract is the effect of distention on uterine secretion. Shih, Kennedy & Huggins (93) were not primarily interested in this point, but their data carry a very definite suggestion that uterine secretion, like that of the intestine, is augmented by a rise in tension. In all animals studied the secretion rates were higher in closed uterine segments than in open fistula.

In no other place is a consideration of intra-uterine tension of greater significance than in the study of the factors governing the onset of labor. One of the most provocative studies on this subject is that of Reynolds & Foster (82). These authors measured mean intra-uterine pressure at the center of the fetal sac on the twenty-second, twenty-eighth, and thirty-first days of pregnancy in the rabbit. The data show a temporary fall in mean pressure between days twenty-two and twenty-eight with a subsequent rise on day thirty-one. At this time the pressure was not significantly different from the level on the twenty-second day, i.e., between 3 and 4 cm. of water. From these objective measurements and from the dimensions of the intra-uterine mass, Reynolds & Foster estimate that the tension on the uterine wall at the placental attachment (top, or

mesial) remains very close to zero (atmospheric) until after the twenty-eighth day when it rises to approximate the tensions calculated for the lateral and antimesial (lowermost) uterine walls. In the words of these workers, "The intrauterine pressure is the local determiner of the efficiency of the flow of maternal blood through the uterus.—The onset of labor in the rabbit coincides with a degree of uterine distention which imposes on the mesial wall, i.e., placental vascular bed of the uterus, tension about equal to the maximal tension occurring elsewhere on the uterine wall."

The assignment of a dominant role to the changes in intra-uterine tension would extricate us from some of the difficulties inherent in any attempt to explain parturition simply on a hormonal basis. Unfortunately, apposite data from another kind of experiment do not endorse such an assignment. In all species so far studied the length of gestation is not altered when the fetuses are removed and only the placentae are left *in utero*. The recent report of Van Wagenen & Newton (101) now adds the monkey to this list. If tension on the uterine walls were a crucial factor in governing the length of gestation, parturition should be delayed under these conditions since the increasing uterine distention toward the end of pregnancy is almost entirely due to the rapid growth of the fetus. The placenta shows very little growth and fetal fluid actually decreases. Indeed, contrary to the anticipated effects of uterine distention are the results of Haterius (51). In ovariectomized animals bearing only one fetus with its placenta, distention of the remainder of the uterus with inert pellets prevented the abortion which would otherwise have followed shortly after the ovariectomy.

A critical re-examination of the paper of Reynolds & Foster shows the burden of the argument to rest almost entirely upon the different values allotted to the factor of hydrostatic pressure. It may be recalled that the values for uterine wall tension were estimates calculated from objective readings of mean intra-uterine pressure and the dimensions of the intra-uterine mass (wall tension is essentially a function of pressure times the radius of the distending mass). No change in either of these factors could account for the equalization of uterine wall tension reported for the end of gestation. This equalization in the estimated tensions depends on the making of an appropriate allowance for hydrostatic pressure in the calculations for the twenty-eighth day and making no allow-

ance for hydrostatic pressure in the calculations for the thirty-first day. Reynolds & Foster contend that intra-uterine fluid has decreased to such an extent by the thirty-first day that hydrostatic forces need not be considered. This contention is not in harmony with measurements of intraperitoneal pressures. Although the amount of fluid in the abdominal cavity is so small as to be negligible the intraperitoneal pressures were very definitely affected by hydrostatic forces (61, 76).

Even if future work credits the factors of hydrostatic pressure and uterine wall tension with somewhat less importance we shall reap only profit from having our attention focused upon the circulatory changes at the placental site. During pregnancy the entire vascular system is subject to some influence not operative in the nonpregnant animal. Pregnant rats are much more resistant than nonpregnant rats to injections of renin, the pressor extract of the kidney (49). Aortic constriction sufficient to produce mild hypertension in nonpregnant rabbits was found to be without influence on the blood pressure of pregnant animals (28). In the latter animals the pressure remained at normal levels until shortly after parturition when it rose abruptly. In pregnant rats with experimental hypertension the approach of parturition is heralded by a progressive decline in blood pressure starting some four or five days before the onset of labor (49). Similar results have been reported by other investigators in the dog and rat (9). These vascular changes may be merely incidental to some of the more general metabolic alterations which precede parturition. On the other hand they may point the way to some unrevealed placental influences directly concerned with the termination of pregnancy.

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BACTERIAL CHEMOTHERAPY

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The present review will deal with bacterial chemotherapy in its experimental phases; the literature reviewed will be mainly that published during the period from November, 1938, to September, 1940. In a previous review by the author may be found a discussion of the earlier work on this subject (185). No attempt will be made to discuss the clinical side of the subject or studies with infecting agents other than bacteria.¹ The reader is referred to various monographs and review articles which have appeared (13, 17, 31, 34, 35, 61, 66, 71, 132, 164, 185, 186, 229, 258). Although over a thousand chemical compounds related to sulfanilamide have been prepared for their possible use as bacterial chemotherapeutic agents, only a few which have been extensively studied will be discussed in this review. Sulfanilamide, sulfapyridine (2-sulfanilamidopyridine), and sulfathiazole (2-sulfanilamidothiazole) are those which have been investigated in considerable detail. The important problem of the relation of chemical constitution to antibacterial action is not discussed; at present only qualitative conclusions can be drawn because few comparisons of active compounds have been made in a really quantitative manner. A summary of the sulfanilamide derivatives which have been prepared, with data on their therapeutic activity, can be found in the recent review by Northey (213).

METHODS AND TECHNIQUE

A method for the determination of sulfanilamide and allied compounds is important for experimental work in estimating the drugs in blood, urine, body fluids, and tissues, and for controlling

¹ An attempt has been made to make the bibliography complete for the period and limited field covered. However, due to the War, access to foreign journals has not been satisfactory. Data of an experimental nature included in articles of a predominantly clinical nature may have escaped notice. No discussion of the use of the sulfonamide drugs in urinary infections or in studies *in vitro* in urine has been included.

the dosage of drugs in patients. The methods almost universally used at present are modifications of those first described independently by Fuller (82) and Marshall, Emerson & Cutting (191): the diazotization of the drug and coupling of the resulting diazo compound to form a dye which can be estimated colorimetrically. A new coupling component for sulfanilamide determination has been described by Bratton & Marshall (26) and the application of the method to work with individual mice (0.02 cc. blood for a determination) has been perfected (193). A method based on color production with sodium β -naphthoquinone-4-sulfonate has been described by Schmidt (249) and with *p*-dimethylaminobenzaldehyde by Kühnau (136) and Werner (295).

Most of the methods used by various investigators for assessing the therapeutic value of different drugs in mouse infections under the same conditions, and of the same drug under different conditions, have been subject to errors due to the facts (*a*) that the methods of dosage in use do not maintain a uniform concentration of drug in blood and tissue, (*b*) that the same dosage of different drugs results in different blood concentration-time curves because of differences in absorption, distribution, and excretion, and (*c*) that a suitable therapeutic response is not used in the comparison. Schmidt & Hilles (251, 252) have avoided some of these errors by maintaining a more or less constant blood concentration of drug by dosage spaced at four- or six-hour intervals during the period of therapy. The procedure of Bieter, Larson, Cranston & Levine (11, 12) of incorporating the drug in the powdered food insures a reasonably constant drug ingestion due to the regular feeding habits of mice. Using this drug-diet method, Litchfield, White & Marshall (151, 152, 193), have developed a method for the quantitative evaluation of the effectiveness of chemotherapeutic drugs against streptococcus and pneumococcus infections in mice. The Median Survival Dose (S.D.₅₀) is determined and converted to the Median Survival Blood Concentration (S.B.C.₅₀) by a factor which relates blood concentration to daily drug intake of the drug being tested. By using a standard, one obtains comparative values for the Median Survival Blood Concentration which may be nearly absolute, even though the values themselves are variable.

Powell & Chen (219) have used the method of titrating the culture of infecting organisms against a constant dose of drug. They have determined the Median Lethal Dose (L.D.₅₀) of culture

for both control and treated mice. This method appears to warrant further exploration; with modifications, it might be used to obtain quantitative comparisons of drugs.

TREATMENT OF EXPERIMENTAL INFECTIONS

Streptococcus infections.—The use of mice infected with a highly virulent culture of a group-A β -hemolytic streptococcus is still the most common method used for testing the chemotherapeutic activity of new compounds. Most of the comparisons of the therapeutic activity of two or more drugs which have been attempted give more of a qualitative than a quantitative comparison because of inadequacy of the methods used. Lockwood & Robinson (159) in a comparison of the activities of sulfanilamide and benzylsulfanilamide emphasize the importance of such factors as the strain of organism and its animal virulence, the size of the infecting dose, the amount of drug given, the interval between doses, and the duration of the period of therapy. Marshall, Litchfield & White (193) in a comparison of sulfanilamide, sulfapyridine, and diaminosulfone show that some of the above factors can be taken into account by using activity ratios based on Median Survival Blood Concentrations; however, no data are available as to the influence of using different strains of streptococci on the activity ratios.

A few reports have appeared on the treatment of experimental streptococcus infections in rabbits (59, 133, 288), but no significant advance has resulted from experimental infections in animals other than the mouse. The remarkable increased efficiency of serum combined with sulfanilamide as compared with sulfanilamide alone in the treatment of infected mice, reported by Loewenthal (160), could not be confirmed by Colebrook & Maxted (51).

Pneumococcus infections.—Following the report by Whitby (299) that sulfapyridine is a much more effective agent than sulfanilamide in the treatment of experimental pneumococcus infections in mice, numerous articles appeared on the treatment of such experimental infections with this drug (11, 12, 19, 52, 53, 91, 111, 167, 219, 223, 224, 252). A survey of these communications shows widely conflicting results on the effectiveness of sulfapyridine therapy in the mouse; some investigators have found a survival of 100 per cent of the treated mice, while others have reported little or no curative action and merely a prolongation of life. Some of

the reasons for these discrepancies appear to be the use of (a) different strains and types of pneumococci, (b) different methods of inoculation (intraperitoneal or subcutaneous), (c) different amounts and schedules of dosage, and (d) different periods for observations of the untreated mice. Studies of the sensitivity of different types and of strains of the same type have been reported (163, 174, 175, 219, 252, 254, 255, 299). The most extensive studies of the response of different types and strains of pneumococci to sulfapyridine are those of Schmidt and his co-workers (252, 254, 255). These investigators, working with strains of types I, II, and III, found that sulfapyridine was uniformly more effective against experimental infections with type I than against infections with type III, and that infections with certain type II strains responded as did type I, whereas infections with other strains of type II responded as did type III. They suggest that these differences in type and strain response are related to differences in antigenicity. It is now quite clear that certain strains of pneumococci are extremely resistant to sulfapyridine therapy and that others are extremely susceptible; and, in addition, it appears to the reviewer from the evidence available that as much variability in therapeutic response may occur between strains of certain types as between types. The results reported suggest that susceptibility of different strains to sulfapyridine is paralleled by the susceptibility of these same strains to sulfanilamide and sulfathiazole (174, 252).

Whitby (299) found that mice infected with a type I pneumococcus and treated with sulfapyridine were immune, on recovery, to subsequent infection with this pneumococcus. Conflicting data on this immunity are reported by others (37, 147, 161, 253). An explanation of these discrepancies is given by Schmidt & Hilles (254) who showed that the immunity of sulfapyridine-treated mice to reinfection depends to a great extent on the strain of pneumococcus used as the infecting agent. In pneumococcus infections of mice, rats, and rabbits, a combination of antiserum or vaccine with sulfapyridine is reported to be better than the drug alone (95, 124, 175, 176, 220, 221).

The impression obtained from early work that sulfanilamide has little therapeutic value in pneumococcus infections in mice has been corrected by the very careful study of Schmidt & Hilles (251). These authors used infections with forty-seven strains of the thirty types of pneumococci; they found that the therapeutic ef-

fectiveness of sulfanilamide was considerable, but varied with the type of pneumococcus used as the infecting agent. They administered the drug every six hours day and night, maintaining a blood concentration of 8 to 12 mg. per cent, and showed that infrequent administration was responsible for the failure of earlier workers to obtain beneficial effects. Sulfathiazole, recently introduced (79, 174) as a substitute for sulfapyridine in the treatment of pneumonia, has been found to have about the same activity as sulfapyridine in pneumococcus infections in mice (7, 152, 165, 174, 198). Hydroxyethylapocupreine is stated to be as effective as sulfapyridine in a type II pneumococcus infection of mice (24), but was found by other observers (91) to be less effective in an experimental type I infection.

The effectiveness of sulfanilamide and sulfapyridine is reported upon by various observers in pneumococcus infections in rats (54, 94, 95, 125, 220, 221, 222), in rabbits (107, 137, 222) and in dogs (92, 93).

Other bacterial infections.—Although the sulfanilamide group of drugs was first thought to have a specific action on the streptococcus alone, it has now been proved that these chemicals have some effect in a wide variety of experimental infections in animals, and in various infectious diseases in man. A number of papers have appeared during the period covered by this review on the treatment of experimental infections other than those caused by the streptococcus and the pneumococcus. Branham (25) has reported a detailed study of the effect of sulfanilamide and sulfapyridine, with and without serum, on meningococcus infections in mice. She found a combination of either drug plus serum to be more effective than either agent alone, and sulfapyridine better than sulfanilamide. She also found individual strains to vary greatly in their response to both drug and serum. Strains resistant to one drug were also resistant to the other. It has also been found (1,240) that sulfanilamide plus serum is more effective than either agent alone in meningococcus infections in mice. In staphylococcus infections in mice, sulfathiazole has been found to be more effective than sulfapyridine and the latter to be more effective than sulfanilamide (6, 21, 22, 32, 60, 196, 225). The newer reports of the effectiveness of the treatment of experimental tuberculosis in the guinea pig and the rabbit with sulfanilamide or sulfapyridine are still discordant (5, 14, 56, 69, 78, 87, 90, 120, 134, 136, 237, 238,

277). The most extensive and satisfactory papers are those of Follis & Rich (78) and Birkhaug (14). These workers found a decided inhibitory effect of sulfanilamide on the development of the disease. Follis & Rich satisfactorily explain the negative results of certain investigators as due to lack of adequate dosage, improper spacing of doses, and insufficiently long duration of administration of the drug. Steinbach & Duca (278) were entirely unable to confirm the observation of Climenko (50) that N¹-dodecanoylsulfanilamide inhibited the development of tuberculosis in the guinea pig although an exact repetition of Climenko's experiments was carried out.

Sulfanilamide (or allied compounds) has been shown to be effective in the treatment of experimental infections of mice and chickens with certain members of the *Pasteurella* group, including the plague bacillus (38, 39, 63, 65, 86, 259, 272); to favorably influence the course of experimental brucellosis in the guinea pig (48, 103, 202, 305, 306) and mouse (64, 131); to have an effect on the course of anthrax infections in mice (57, 194); and to influence favorably *Hemophilus influenzae* (217), *Listerella* (218), *E. coli* (55, 198), and *S. paradysenteriae* (239) infections in mice, and *Bact. necrophorum* infection in rabbits (108). These drugs appear to be less effective than previously reported in experimental gas bacillus infections (109, 123, 206, 269, 279). Two further papers indicating some effect on *E. typhi* infections in mice have appeared (36, 135). No protective effect of the sulfanilamide drugs has been found in mouse infections due to *P. aeniginosa* (55), *Erysipelothrix* (218), *C. diphtheriae* (8, 241), *H. pertussis* (97), or *Bartonella muris* (68).

Sulfanilylguanidine, which is effective in experimental streptococcus and pneumococcus infections, has been studied by Marshall, Bratton, White & Lichfield (188). This drug which is fairly water-soluble, is poorly absorbed from the intestinal tract and is therapeutically active against various bacteria. The properties of such a drug allow the attainment of a high concentration of drug in the intestine and a low concentration in the blood and tissues, a situation somewhat analogous to the use of sulfanilamide derivatives as urinary antiseptics. It is suggested that this drug or some other having similar properties may prove useful in the treatment of bacterial infections which are mainly or entirely localized in the intestine.

TOXICITY

The toxic effects of repeated administration of large doses of sulfanilamide and sulfanilylsulfanilamide to rabbits and chickens are reported upon by Rosenthal (244). The symptoms presented by many of the chickens suggested peripheral neuritis, but it was not possible to establish this condition on a pathological basis (208). Sulfanilamide, given for long periods to rats with nephrotoxic nephritis, did not affect the course of the experimental disease (271). The toxicity of sulfanilamide for the fish, frog, and chicken is reported (150). The drug appears to be most toxic for the fish and least for the frog, while excretion in all three of these lower vertebrates is much slower than in mammals. Speert (273) has shown that the prolonged administration of sulfanilamide to pregnant rats produces deleterious effects upon the offspring, such as increased intra-uterine and postnatal mortality, decreased litter size, diminished birth rate, and selective stunting of growth. The observation of James (114, 115) that administration of sodium acetate along with sulfanilamide decreases its acute toxicity for mice, in spite of the fact that acetylsulfanilamide is more toxic than the parent derivative, is of interest.

The acute toxicity of sulfapyridine administered by mouth appears to be much less than that of sulfanilamide (204, 303), but this is due to poor absorption of the sulfapyridine, and the Median Lethal Dose cannot be determined in mice (167, 192, 219). By the use of a soluble sodium salt of sulfapyridine it has been shown that on the basis of blood concentration this drug is more toxic acutely than sulfanilamide (192). No information is available as to the chronic toxicity of sulfapyridine based on a maintained and known blood concentration of the drug. Studies have been made of the effect of repeated peroral administration of one dose per day (204, 219). These have shown no toxicity except that resulting from the deposition of acetylsulfapyridine in the form of concretions and stones in the genito-urinary tract of rats (98) and rabbits and monkeys (204). Reports on the pathological changes following administration of sulfapyridine describe the urinary concretions and degenerative changes in the epithelium of the kidney tubules (2, 284). Clinically, sulfapyridine appears to be considerably more toxic than sulfanilamide (30).

Sulfathiazole has been shown to be less toxic acutely than sulfapyridine for mice (169, 289); its chronic toxicity for mice is

greater than that of sulfapyridine, although it is stated to have less chronic toxicity for rats and monkeys (289). In animals other than the dog, the evaluation of chronic toxicity of these drugs is complicated by the occurrence of acetylation.

A slowly developing anemia can be produced in dogs and monkeys (215), rats (171), and mice (231) by the repeated administration of sulfanilamide. On the basis of equal dosage by mouth, sulfanilamide was found to be more toxic than neoprontosil, which in turn was more potent than sulfapyridine in reducing the red cell count of rats (172); on the basis of the minimal effective blood concentration which is necessary to produce a significant decrease of red cells in mice, sulfapyridine was one half as toxic as sulfanilamide, whereas diaminodiphenylsulfone was about twice as toxic as sulfanilamide (231). Cyanosis is reported in rats (171, 293), in mice, and in chickens (232) from the administration of sulfanilamide. This cyanosis may be associated in rats with methemoglobin, sulfhemoglobin, or an unidentified pigment (293), in mice with sulfhemoglobin, and in chickens with methemoglobin (232). James (116) found that pigments are obtained by the oxidation of sulfanilamide which are absorbed by the red cells and alter the color of blood without affecting the oxygen capacity or spectrum. Rimington has amplified his investigation of the porphyrin excretion after sulfanilamide to include other drugs of the sulfonamide series and simpler related chemical substances. The chemical grouping necessary for increasing porphyrin excretion is defined in the same terms as the structure causing methemoglobin formation; namely, an aromatic amino group, unsubstituted or potentially free (234). Sulfapyridine was found to be no more toxic than sulfanilamide in regard to its porphyrinuric action (235).

ABSORPTION, EXCRETION, AND DISTRIBUTION

It has been shown that in man sulfanilamide is absorbed from the large intestine and from the rectum (287). Although certain investigators (3, 102) conclude that sulfapyridine is absorbed very rapidly from the gastrointestinal tract of animals and human subjects, the results of several others indicate clearly that the absorption of sulfapyridine is slower, less complete, and more variable than is that of sulfanilamide under the same conditions (168, 192, 219, 256, 281). Sulfapyridine, like sulfanilamide, is absorbed very slowly and to a limited extent from the stomach, but very rapidly

from the intestine (192). Scarcely any data can be found on the absorption of sulfathiazole in animals, but in man it appears to be absorbed more completely and less erratically than sulfapyridine (230, 247).

Sulfapyridine is excreted similarly to sulfanilamide by the kidney of dogs (122), while sulfathiazole appears to be excreted much more rapidly (169, 289). All of the sulfanilamide derivatives which have been examined are found partly conjugated in the urine of man and of all animals other than the dog and frog. This conjugated derivative, in the case of sulfapyridine (3, 187, 227, 281) and of sulfathiazole (289) has been proven, like that of sulfanilamide, to be the acetyl derivative. Further evidence which shows that the liver is the site of acetylation of sulfanilamide in the rabbit is presented by Stewart, Rourke & Allen (280) but Van Winkle & Cutting (290) find that in cats an extrahepatic acetylation may occur with sulfapyridine. Although eliminated mainly by the kidneys, sulfanilamide (10, 45, 304) and sulfapyridine (70, 219) are excreted in the secretions of the digestive glands; the amounts eliminated by this route are extremely small.*Sulfanilamide is also excreted in human milk (101, 216). There are some observations that indicate the formation of a phenolic compound from sulfanilamide or sulfapyridine by the animal body, but the evidence does not appear to be conclusive (116, 117, 260, 264). Neither sulfanilamide nor sulfapyridine appears to be methylated in passing through the body (118). On the other hand, the N⁴-methylsulfanilamide is demethylated (148).

Sulfapyridine resembles sulfanilamide in its ready penetration to all tissues and body fluids in a concentration not far removed from that in the blood (15, 46, 49, 192); unlike sulfanilamide, it is usually present in higher concentration in the liver than in other tissues (192). Sulfapyridine passes into the spinal fluid in a manner similar to that of sulfanilamide (67, 192). Sulfathiazole, on the other hand, penetrates only very slowly into the spinal fluid (247).² It is important to realize that all sulfanilamide derivatives do not readily pass from the blood to the spinal fluid (189, 291). While the blood plasma contains somewhat less sulfanilamide than the corpuscles (104, 270), sulfapyridine is present in slightly higher concentration in the plasma than in the corpuscles (105, 192).

* Also, unpublished observations from this laboratory.

MECHANISM OF ACTION

In a consideration of the mechanism of action, three factors are involved: the drug, the infecting bacteria, and the host. The theories which have been proposed may be said broadly to involve one or more of the following propositions: (a) the drug stimulates certain defense mechanisms of the host; (b) the host changes the drug into a bactericidal substance; (c) the drug neutralizes the toxic bacterial products; and (d) the drug acts directly on the bacteria, either killing them or interfering with their ability to multiply. Since all investigators now agree that the mechanism of action involves to some extent a direct effect of the drug on the bacteria, further elucidation of the problem necessitates an explanation of just how the drug adversely affects the bacteria. We shall assume that the mechanism of action is identical in the case of different species and strains of bacteria and in the case of different sulfanilamide derivatives.

Most of the numerous papers on the mode of action of these drugs have been concerned with studies *in vitro*. We shall discuss these first in a general way, and later such studies *in vitro* as bear on theories of the nature of the deleterious action of these drugs upon bacteria.

Marked discrepancies are apparent in the reports of different investigators as to the effect of these drugs on bacteria *in vitro*; some workers find no effect, others a definite bactericidal effect. These discrepancies are due in the main to differences in the conditions of the experiments (185). We now know that the species and strain of organism used, the size and age of the initial inoculum, the composition of the medium, and slight changes in the temperature at which the test is performed may have a marked effect on the result obtained. The effect of temperature has been studied especially by White (301, 302) and confirmed by others (210, 274, 294). Further experiments on the effect of changes in the medium are described by Bliss (18). Lockwood's observations on the effect of peptone in neutralizing the action of sulfanilamide have been extended (158). Weld & Mitchell (294), in their experiments, do not find support for the idea that peptone *per se* interferes with the bactericidal effectiveness of sulfanilamide; they believe that peptone furnishes a richer medium for growth. It has been stated by many investigators that the poorer the medium used for the

growth of the organisms, the more effective is sulfanilamide (83, 143). Although this assumption probably contains a great deal of truth, it does not completely explain the effect of changes in the medium on the activity of sulfanilamide. Wolff & Julius (307) show that streptococci in broth medium, where growth is extremely slow, are not influenced by sulfanilamide, but a change to a blood-broth medium, where better growth occurs, results in bacteriostasis. They conclude that a certain rapidity of growth is essential for the action of sulfanilamide. The finding that substances which neutralize the inhibitory action of sulfanilamide on bacteria (anti-sulfanilamide factors) can be obtained from bacteria (74, 89, 276), peptones, various animal tissues (178, 276, 308), red blood cells and urine of the mouse (83), and yeast (296, 308), and the statement that purified preparations, while retaining their antisulfanilamide potency, have no effect upon growth necessitate a re-examination of the effect of changes in medium on the effectiveness *in vitro* of the sulfonamide drugs. These important investigations will be discussed later as to their bearing on the mechanism of action of these drugs.

Most of the studies *in vitro* have been carried out with sulfanilamide and streptococci. However, experiments *in vitro* have been made with the pneumococcus (20, 73, 75, 112, 165, 167, 173, 175, 188, 198, 228) the staphylococcus (20, 138, 165, 198, 225, 226, 233, 274), the gonococcus (121, 181, 198, 298), the meningococcus (181, 257), the tubercle bacillus (4, 16, 20, 50, 88, 236, 238), the enterococcus (209, 211), the typhoid-dysentery group (139, 149, 188), *E. coli* (198), the tetanus bacillus (198), *Haemophilus influenzae* (100), the diphtheria bacillus (212, 242), and some aerobic sporogenic bacilli (243). These experiments, as in the case of sulfanilamide and the streptococcus, give varying results due to the same factors which have been described above. Aside from information which they may give concerning the mechanism of action, these experiments *in vitro* may be valuable as an indication of the activity of different drugs upon different strains of various bacteria *in vivo*. However, very little information is available concerning the relation between activity *in vitro* and *in vivo*. The information available on this question (141, 180, 214, 282) is in general contradictory and insufficient to give a definite answer. However, it appears that no correlation exists between the effectiveness *in vivo*

and *in vitro* of sulfapyridine against different strains and types of pneumococci (163, 255).

In regard to streptococci, it was stated early that sulfanilamide prevented the formation of capsules, thus rendering virulent organisms susceptible to phagocytosis, but this was not confirmed, although morphological changes in the streptococci have been described (185). Whitby (299) reported a degeneration and final disappearance of the capsule of pneumococci in the peritoneum of sulfapyridine-treated mice. Levaditi, Vaisman & Krassnoff (143) stated that the pneumococci in the peritoneal cavity of sulfapyridine-treated mice fail to form capsules, and Telling & Oliver (283) stated that in the human subject sulfapyridine so alters the capsule as to make typing impossible. Subsequent observers (33, 76, 112, 170, 176, 228) have been unable to confirm these observations.

Several workers (47, 72, 285), have shown that sulfanilamide or neoprontosil, *in vitro* in a concentration of 10 or 100 mg. per cent does not promote phagocytosis. On the other hand, they find that more dilute solutions of these drugs do promote phagocytosis of streptococci. Tunncliffe (286), using *Streptococcus viridans*, found stimulation of phagocytosis with sulfapyridine concentrations of 1 to 100 mg. per cent, while Reid (228), using pneumococcus, obtained no effect on phagocytosis with a concentration of 10 mg. per cent. This effect is ascribed to a stimulation of the leucocytes and not to any opsonic action on the bacteria. King (126) and Henschel (110) found in tissue cultures that sulfanilamide and neoprontosil stimulate the rate of leucocyte migration. These drugs do not appear to affect the quantity or speed of production of specific antibodies (173). Certain other authors (33, 144, 201) have found phagocytosis to be increased by these drugs, but this can be ascribed to the effect of the drug on the bacteria. The fact that in the treatment of pneumococcus infections in the mouse phagocytosis appears to play a much less important role than in streptococcus infections minimizes the importance of the above-described stimulation of leucocytes as a factor in the mode of action of the sulfonamide drugs (162). Domagk (62) in a recent publication states that some sulfanilamide derivatives act *in vivo* only, apparently by stimulating phagocytosis and other defense mechanisms, while others exhibit a bacteriostatic action *in vitro*; no evidence is given, however, for this view.

Many reports have appeared in the literature to the effect that the sulfonamide drugs neutralize the toxic products of bacteria or prevent their formation. Thus Carpenter and his co-workers have reported an antitoxic effect of sulfanilamide and some of its derivatives on toxins formed by the gonococcus, pneumococcus, staphylococcus, streptococcus, *Cl. botulinum*, *Cl. tetani*, *Cl. septicum*, and *Cl. perfringens* (40, 41, 42, 43, 44). Levaditi and co-workers found certain sulfonamide derivatives to possess an anti-endotoxic action against intoxication by the endotoxins of the Flexner dysentery bacillus and the gonococcus (142, 145); Mayer found that sulfanilamide and sulfapyridine neutralized tetanus toxin *in vitro* but not *in vivo* (197). On the other hand, a number of investigators (58, 85, 96, 113, 203) believe that sulfanilamide has no effect on the soluble toxins of the streptococcus once they are formed. Gross, Cooper & Lewis (99) could not demonstrate any anti-endotoxic action of sulfanilamide against formalin-killed meningococci in mice, against formalin-killed streptococci in mice or guinea pigs, or heat-killed streptococci in guinea pigs. King and co-workers (128, 129) in tissue culture studies have found that the effect of sulfanilamide on the production or neutralization of streptococcal hemolysin is secondary to the bacteriostatic effect; but, on the other hand, neoprontosil decreased the hemolytic effect of streptococci without producing bacteriostasis. Bayliss (9) and Rigdon & Freeman (233) were entirely unable to confirm the observation that the sulfonamide drugs neutralized staphylococcus toxin either *in vivo* or *in vitro*. It is difficult to assess these discordant results on the antitoxic properties of the sulfanilamide group of drugs in relation to their mode of action. However, since it appears fairly certain that an antitoxic action does not play any role in the effect of sulfanilamide upon streptococcus infections, it is doubtful if this possible antitoxic effect is important in the explanation of the mode of action of these drugs.

It appears from the literature summarized above that the sulfonamide drugs act directly upon bacteria by inhibiting their multiplication. Under certain conditions this leads to sterilization of the culture; no satisfactory evidence has been adduced that stimulation of defense mechanisms of the host or direct interference with the production or action of toxic products formed by the bacteria plays an essential role in the mode of action. Since there is evidence that inhibition of growth of the bacteria occurs in infected

animals (166, 300), one can assume for the present that the direct action of these drugs is upon the bacteria and that such other factors as may be necessary for the cure of an infected animal are secondary to this action.

That the curative effect is not simply a destruction of the invading bacteria, as has been shown to occur under certain conditions *in vitro*, is suggested by several pieces of evidence. Sulfanilamide added in the same concentration to various samples of human blood infected with streptococci and incubated under the same conditions produced effects varying from slight bacteriostasis to a bactericidal effect. The samples of blood which showed a bactericidal effect were those containing natural antibodies (122). Also, sulfapyridine plus specific immune serum is more effective on pneumococci, *in vitro*, than either agent used separately (33, 75, 275), and, as mentioned above, there is a synergism between sulfapyridine and serum in pneumococcus mouse infections. Strains of types I and III pneumococcus were equally susceptible to sulfapyridine *in vitro*, but exhibited a marked difference in their response *in vivo* (255). The response to therapy with sulfapyridine in infected mice parallels the immune response elicited by these types (176).

Two main types of host response have been described. With streptococcus infections, phagocytosis seems to play an important role, while specific antibody formation is of minor importance. On the other hand, with drug-treated pneumococcus infections, phagocytosis is slight and the naturally developing immune bodies are able to cope with the infection after the drug has exerted its effect (162). Additional evidence of the different host response in streptococcus and pneumococcus infections is found in the fact that two strains of mice exhibit a marked difference in the S.D.₅₀ of sulfanilamide with a streptococcus infection, but show no difference with sulfapyridine in a pneumococcus infection.³

From the above discussion, it is obvious that we must consider the therapeutic action of sulfanilamide and allied drugs to be primarily an effect upon the bacterial cells.

Before discussing the theories which have been proposed to explain the action of these drugs, we must mention "drug-fastness." Well-known in the case of protozoa and various chemotherapeutic drugs, this phenomenon has only recently been described

³ Unpublished observations from this laboratory.

for bacterial chemotherapy. MacLean, Rogers & Fleming (175) showed that pneumococci can, in an infected mouse treated with sulfapyridine, readily establish a tolerance or fastness to the drug. This observation has been confirmed and "sulfapyridine-fastness" has been demonstrated both *in vivo* and *in vitro* (177, 179, 207, 250). MacLeod (177, 179) has made a preliminary comparison of the "drug-fast" and parent strains. No changes in morphology, type-specificity, or virulence were noted with the induced "fastness," but acquisition of "fastness" was associated with a marked diminution in the production of hydrogen peroxide in cultures and with a marked loss of dehydrogenase activity for certain three-carbon compounds. It is stated that the gonococcus and meningococcus can be made fast to sulfapyridine (297), the gonococcus to sulfanilamide (23), and the diphtheria bacillus to sulfanilamide (212).

There remains for discussion the important problem of how these drugs affect the bacteria, inhibiting their multiplication. In 1937, Mayer (195) advanced the hypothesis that the activity of sulfanilamide was not due to this substance itself, but to some oxidation product; he examined certain oxidation products and believed that *p*-hydroxaminobenzenesulfonamide was responsible for the antibacterial action of sulfanilamide. Shaffer (262, 263) has strongly advocated this oxidation theory of the action of sulfanilamide and writes

that sulphanilamid and sulphapyridine are not themselves bactericidal and that their therapeutic (and toxic) effects are due to oxidation products of these substances formed by atmospheric oxygen under the catalytic influence of respiring tissues or organisms.

Fox (80) believes that sulfanilamide is oxidized during therapy, but his evidence is not at all conclusive. James (116) has isolated from the urine of patients receiving sulfanilamide two substances which correspond in their properties to the acetyl derivative of *p*-hydroxaminobenzenesulfonamide and of *p*-aminophenol; and Rosenthal & Bauer (246) claimed to have developed a method which demonstrated this hydroxyamine in the urine of animals given sulfanilamide but later (245) stated that the reaction they used was not specific. Harris (106) has shown that upon the interaction of certain tissues with sulfanilamide *in vitro* an oxidizing agent is formed which can convert hemoglobin to methemoglobin.

Bratton, White & Marshall (27) prepared pure *p*-hydroxaminobenzenesulfonamide, but found no evidence that this was the active substance in sulfanilamide therapy; if it is the active component, one must assume that it is formed in or upon the bacterial cell. One of the arguments used to support the theory that an oxidation product of sulfanilamide is responsible for activity is the statement that this drug is inactive under anaerobic conditions (80, 262, 263), but many observers have found that it is active under these conditions (28, 29, 162, 267). It has been found that the oxidation-reduction potential of control bacterial cultures remained elevated until active multiplication was well advanced, then rapidly fell, while with sulfanilamide cultures the potential remained elevated much longer until bacteriostasis ceased (81, 292). It is difficult to interpret these observations; they may merely represent inhibition of growth by sulfanilamide.

✓ Another hypothesis, which can be considered an extension of the oxidation theory, is that proposed and sponsored by Mellon and his co-workers in a long series of papers (153, 154, 155, 182, 183, 184, 199, 200, 265, 266, 268). These investigators report that sulfanilamide and allied compounds possess a slight degree of anticalase activity and that ultraviolet radiation markedly increases this activity. They further assume that an oxidation product, *p*-hydroxaminobenzenesulfonamide, acts as an anticalase and allows hydrogen peroxide to accumulate; this injures the bacteria and prevents growth. Long & Bliss (164) point out that streptococci vary enormously in their ability to produce peroxide, but there is no correlation between their production of peroxide and their sensitivity to sulfanilamide *in vitro*. Fuller & Maxted (84) also found that sulfanilamide is very effective against strains of streptococci which do not produce peroxide.

The last theory to be discussed is the one which, in the opinion of the reviewer, offers the most promising basis for further work. Levaditi (140) assumed that the drug, or "the active principle X" formed from it, interferes with the bacteria's source of nitrogen. Independently, Lockwood (156, 158), on the basis of the inhibiting action of peptone on the effectiveness of sulfanilamide and sulfapyridine *in vitro*, proposed a similar theory, namely, that the drug interferes with the nutritional requirements of the bacteria. He thought that this effect might be achieved by preventing the organism from utilizing the protein substrate. The addition of pep-

tone (protein split products) to media such as serum supplies an excess of easily assimilable nitrogenous material and hence inhibits the bacteriostatic action of the drug. On this basis, he explains the fact that the presence of necrotic tissue or *débris* diminishes the effectiveness of sulfanilamide clinically (157). Stamp (276) was able to prepare from broth cultures of streptococci a fraction capable of antagonizing the bacteriostatic action of sulfanilamide and sulfapyridine *in vitro*. This fraction, which was active in low concentrations, and resistant to heat, dilute acids, and alkalis, appeared to be nonprotein in nature and to consist of substances of relatively low molecular weight, including free amino acids. Green (89) obtained a preparation, a growth-stimulating or "P" factor from *Brucella abortus*, which antagonized the action of sulfanilamide *in vitro*. Woods (308) found that yeast extracts contained a substance which, like the substances prepared by Stamp and Green, abolished the inhibitory action of sulfanilamide and sulfapyridine on the growth of bacteria. A survey of the chemical properties of purified fractions containing the antisulfanilamide factor, as well as its behavior in growth tests, suggested that it might be chemically related to sulfanilamide. Since the chemical evidence showed that the factor contained an amino group and an acidic group, *p*-aminobenzoic acid was examined and found to have high antisulfanilamide activity. Strong circumstantial evidence was then obtained that *p*-aminobenzoic acid is the antisulfanilamide factor present in yeast, bacteria, and tissues, although isolation of the factor from yeast has not yet been accomplished. Selbie (261) reports that *p*-aminobenzoic acid antagonizes sulfanilamide *in vivo* in the treatment of mice infected with streptococci.

On the basis of the above results, Woods has proposed the following hypothesis regarding the mode of action of sulfanilamide. Taking *p*-aminobenzoic acid to include the probably related, if not identical, antisulfanilamide factor that occurs naturally, it is suggested that *p*-aminobenzoic acid is essential for the growth of susceptible bacteria. This essential metabolite is normally synthesized by certain bacteria. The enzyme reaction involved in the further utilization of *p*-aminobenzoic acid is subject to competitive inhibition by sulfanilamide. Since the antisulfanilamide factor appears to be widely distributed in small amount, this may account for the failure of sulfanilamide in complex media. In case the organ-

ism is able to make sufficient *p*-aminobenzoic acid to overcome the competitive inhibition, growth occurs; this is the case with organisms insensitive to sulfanilamide. Variations in media and conditions of the experiments may affect the amount of *p*-aminobenzoic acid formed by the organisms and hence the response to sulfanilamide.

MacLeod (178) has demonstrated an antisulfanilamide factor in all "peptones" examined (except Witte's), in enzymatic hydrolyzates of casein, and in extracts of fresh muscle, pancreas, and spleen. An acid or alkaline hydrolyzate of casein did not contain the factor. None was present in fresh liver or urine, but on hydrolysis the antisulfanilamide factor appeared to be formed. It was found to be present in pus and in some serous effusions, but not in blood serum. In certain species of bacteria it was demonstrated to be present in the supernatant culture medium, but not in the bacterial cells; in other species, the reverse was found to be the case. In accord with Woods's theory, certain bacteria insensitive to sulfapyridine appeared to form a larger amount of the factor than certain ones highly sensitive to the drug. Development of drug-fastness in a type I pneumococcus was associated with increase in the production of the inhibiting factor. The author concludes that his inhibiting substance resembles *p*-aminobenzoic acid in some respects, but in others it differs from this compound. Chemical isolation and identification of the antisulfanilamide factor or factors would appear to be the next advance to be made. Possibly there are several antisulfanilamide factors of related chemical structure.

In considering how far this attractive theory will explain the other known facts concerning the action of these drugs, it must be remembered that other factors than bacteriostatic or bactericidal action *in vitro* are necessary to make a drug an effective chemotherapeutic agent: low toxicity to the defense mechanism of the host and penetration of all tissues and organs may be just as important as the action of the drug on the bacteria.

In passing, mention may be made of the mode of action of sulfanilamide derivatives. Such derivatives are of two classes: first, a substitution in the aryl amino group, and second, a substitution in the sulfonamide group. Many of the sulfonamide derivatives of the second class, e.g., sulfapyridine, sulfathiazole, etc., owe their activity to the compound as such. However, certain acyl and

hydroxy derivatives of this class are decomposed in the host to sulfanilamide⁴ and may owe part, if not all, of their activity to this substance. In regard to derivatives of the first class, it is known that many of these are changed in the body to sulfanilamide (82, 130, 148, 190, 205). James & Fuller (119) present evidence that the activity of benzylsulfanilamide is due to the sulfanilamide produced from it.

In conclusion, it appears to the reviewer that further work upon the theory proposed by Woods, including isolation of the antisulfanilamide factor or factors, will lead to valuable and far-reaching results in this field. A determination of the true relationship between activity *in vivo* and *in vitro* of different compounds under different conditions with different species and strains of bacteria should also advance our knowledge. A study of the relationship of chemical constitution to therapeutic activity, with comparisons based on equally effective blood concentrations, may change the generally held opinion that there is little or no such relationship.

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⁴ Unpublished observations from this laboratory.

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HISTAMINE AND ANAPHYLAXIS

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DEVELOPMENT OF THE HISTAMINE THEORY OF ANAPHYLAXIS

The pharmacological effects of histamine were discovered in 1910, and Dale & Laidlaw at once stressed "as a point of interest and possible significance" the resemblance to many immediate symptoms of anaphylaxis. In fact, the possibility of histamine as an intermediary was suggested a few months earlier by Biedl & Kraus (17). They had drawn attention to the similarity between anaphylactic and peptone shock in dogs and, later, in guinea pigs, and had concluded that the symptoms of anaphylaxis might result from the presence of a substance identical with that physiologically active in Witte peptone. Popielski had extracted from it a vasodilator substance, vasodilatin, which Biedl & Kraus thought to be the responsible one, and they postulated its identification as the next step of investigation. To-day we know that Popielski's vasodilatin was histamine, which may be present in large amounts in Witte peptone, although it is not the active principle of the peptone proper.

At that time our conception of the mechanism by which toxic substances might be involved in the antigen-antibody reaction was different from what it is today. It was thought that they originated from the antigen or from blood constituents, being probably cleavage products formed by some proteolytic action. Today the evidence points to the tissues as the source from which histamine is derived and to its release, not to its formation, in the antigen-antibody reaction. This change in conception has been pointed out on several occasions by Dale (30, 31, 32). The following summary, taken from his papers, is given because some of the apparently antiquated ideas, and their supporting experimental evidence, may again prove to be of value, although in a new form and with a different meaning.

Vaughan favoured the view that the anaphylactic condition depended on the production of a highly specific enzyme splitting the antigen and liberating from it toxic cleavage products. Friedberger supposed that "the complex of antigen and antibody was formed in

the blood and condensed upon itself a proteolytic ferment"¹ by which an unspecific toxic cleavage product, anaphylatoxin, was formed. It was soon found that serum also became toxic on treatment with various colloids. This so-called anaphylatoxin formation in serum suggested the blood as source of the toxin formation and overcame the difficulties which had arisen from mere quantitative considerations. It was thought that the effect was either the outcome of "some rather vaguely defined disturbance of the colloidal equilibrium of the proteins" or due to enzymatic action of the trypsin present in blood, but normally prevented from acting by inhibitory factors constituting the so-called antitrypsin. According to this view the treatment with colloids would "destroy or weaken the antitryptic power of normal serum liberating the activity of its tryptic ferment and initiating a self-digestion of the serum proteins." When it became established (Schultz, Dale) that the anaphylactic symptoms resulted from the interaction of antigen and antibody in the tissue cells and not in the circulating blood the two alternatives for the anaphylatoxin formation in serum, the physical and proteolytic theory, as Dale had termed them, had to be transferred from the blood to the reacting tissue cells. "The pronounced tendency of histamine to enter into loose combination with colloidal substances" would facilitate the assumption that a change in colloidal equilibrium is sufficient for its release. On the other hand Abel & Kubota suggested an intracellular formation of histamine by protein digestion. As Dale pointed out there could be little doubt that the union of antigen and antibody was actually a prelude of proteolysis, but there was at that time "no convincing evidence, either of an immediate protein digestion resulting from the union of antigen and antibody or of the liberation of histamine in the enzymatic cleavage of proteins."

The present conception was originated by Lewis (92). From the triple response of local anaphylaxis in the human skin, he concluded that the antigen-antibody reaction in sensitized cells represented a special form of cell injury which, like other stimuli injurious to cells, led to release of preformed H-substance. In the same year Best, Dale, Dudley & Thorpe published their experiments on the histamine content of various tissues. The presence of an atropine-resistant vasodilator substance in tissue extracts had long been known and the presence of histamine in such extracts

¹ The quotations are from Dale's reviews.

had been demonstrated from time to time, but the fact that histamine is a normal constituent of many tissues was firmly established for the first time by these experiments, and we may today regard it as the H-substance responsible for the uncomplicated triple response.

The release of preformed histamine does not involve the formation of a toxic substance. Accordingly it might be thought that speculations as to how such a substance may be formed have become superfluous. This is not the case. The expression "cell injury" is susceptible to further analysis and the possibilities discussed for the formation of a toxic substance, the "physical" theory and the "proteolytic" or—to give the latter a wider implication—the "enzymatic" theory, may profitably be applied to this problem. Such a discussion is at present confined to what is known about other mechanisms by which histamine is released. We have to realize that the "injury" may involve, in addition, processes which result in the formation from the tissue constituents of toxic substances, the effects of which may participate even in immediate anaphylactic manifestations. The resemblance between anaphylactic and peptone shock has been mentioned. This resemblance is more complete than that between anaphylactic and histamine shock. In dogs, for instance, the swelling of the liver and the increase in lymph flow from the thoracic duct are much greater in anaphylactic and peptone shock than in histamine shock, while the coagulability of the blood is lost in the former two conditions only, due to liberation from the liver of an anticoagulant substance which is probably identical with heparin (43, 103, 128).

POSSIBLE MECHANISMS FOR THE RELEASE OF HISTAMINE

The cell cannot be regarded as a diffusion-tight chamber in the interior of which histamine is present in a freely diffusible form, and "injury of the cell" cannot simply be interpreted as damage of an outer cell membrane allowing the histamine to diffuse out. This statement might seem at first view to contradict the fact that grinding up small pieces of tissue in saline solution with silica or sand brings histamine into solution. However, this procedure releases only part of the histamine. Trethowie (122) showed that cells may be divided into small particles which retain their histamine. Samples of dog's liver were ground up with silica in saline solution and centrifuged for a short time. Microscopical examination of the

supernatant fluid revealed the presence of some cellular debris which was spun down by further centrifugation and washed. It contained large amounts of histamine which were brought into solution by heating. Feldberg & Kellaway (57) perfused the dog's liver and found that, in the perfusate obtained after an intraportal injection of staphylococcal toxin, there were suspended large amounts of granulated cellular debris, the particles of which were so small that no circulatory effects were produced when the perfusate was injected intravenously into a cat. The particles nevertheless had retained their histamine which was brought into solution and became active when they were coagulated by heating.

The protoplasmic structure of cells has been regarded as consisting of mixed lipoprotein films (104) in which the histamine is anchored. It is not released from this anchorage by a change in potassium concentration which appears to be the physiological stimulus for the release of acetylcholine, nor by other ions or by a change in the pH (112). The release observed in perfusion experiments by distilled water (112) and by alcohol (36) is without significance for the release in anaphylaxis. From the assumption of a lipoprotein structure of the protoplasm, it follows that factors which alter either the proteins or the lipins might cause a release of histamine. The proteins may be coagulated by heat, a change which would lead to a release of histamine. Small pieces of tissue or cell debris brought into boiling saline solution give off their histamine. The release by mercuric chloride demonstrated in perfusion experiments (57, 73) also results from coagulation. The metal combines with the protein of the tissue and coagulates it. Physiologically more interesting are the experiments with trypsin. Rocha e Silva (105, 106), starting from observations on snake venoms, found that crystalline trypsin, the effects of which on circulation and smooth muscles resemble in many details those of histamine, venom, or anaphylaxis, causes a release of histamine from the perfused lung of the guinea pig.² Two different mechanisms may be involved in this action. Digestion of the protein would lead to a destruction of the normal lipoprotein structure with consequent

² The perfusate was extracted according to Code's method. Since the solution contained dustlike particles, which, although without cellular structure, may have retained their histamine, it may have been released from these particles during the process of extraction. Part of the assayed histamine, however, had certainly been free in the solution.

release of histamine. In addition, at one stage of the tryptic digestion, peptones must be formed which would initiate a further release. Such an action of peptone was suggested by Lewis (92) and by Dale (31) and the release has lately been demonstrated in perfusion experiments (58) and during peptone shock (38, 39, 40 121). The fact that a split product of proteolytic digestion releases histamine is interesting because the same property in an even more pronounced degree has to be attributed to a cleavage product of lipin digestion. This mechanism again has been brought to light by analyzing the mechanism of snake venom poisoning.

It has long been known that different snake venoms and bee venom split off oleic acid from lecithin so forming a lytic substance, lysolecithin. Lysocephalin is said to be derived in a similar way.³ Lysolecithin is formed in the tissues of envenomed organs (52, 56). Its formation is responsible for the liberation of histamine observed in perfusion experiments with numerous snake venoms (52, 123) and bee venom (54, 55). Injected into perfused organs it releases histamine. Further cell debris, treated with lysolecithin, is dissolved and the histamine it retained brought into solution (52, 57, 122).

The enzymatic theory put forward to explain the formation of "anaphylatoxin" from the antigen, then from the blood, and still later from cell constituents may thus be applied to explain the release of histamine. If we assume a proteolytic mechanism, the antigen might affect the sensitized cells by destroying or weakening antitryptic factors and thus allowing the cellular trypsin to act. Simultaneous formation of peptones could account for the greater resemblance of peptone to anaphylactic shock, without invalidating the histamine factor in these phenomena. The assumption of a splitting of the tissue lipins would have to deal with the fact that there is at present no evidence for a lytic effect in normal anaphylaxis. The absence of signs of haemolysis, however, does not exclude intracellular formation of lysolecithin, and if it were to occur in a small degree it could hardly be detected by examining tissue extracts for their haemolytic power. The haemolytic effect of small amounts of lysolecithin is masked by the antihaemolytic property of tissue extracts (56). At its best, a diminution of this property could be expected. The original failure to detect the formation of lysolecithin in tissue extracts incubated with venom (14) may have

³ The term lysocithin is used for the mixture of both substances.

resulted from a disregard of this factor. We shall see that, in addition to lysolecithin, a substance acting on smooth muscle is formed by the actions of venoms on samples of lecithin and that the effects of this substance are such as would explain certain discrepancies existing between histamine and anaphylactic responses of smooth muscles.

Enzymes accelerate normally occurring reactions and consequently disturbances in the colloidal equilibrium could result in changes similar to those produced by the enzymes or could alter the lipoprotein structure in such a way as to free the histamine. Thus the old alternative between the "physical" and "enzymatic" theory still has to be reckoned with.

EVIDENCE FOR LIBERATION OF HISTAMINE IN ANAPHYLAXIS IN VARIOUS SPECIES

Dogs.—The liver can be regarded as the organ most clearly involved in shock in these animals. Under normal conditions of sensitisation its removal prevents the occurrence of shock on the reinjection of serum. The histamine equivalent (as chloride salt) per gram of fresh liver of an adult dog varies between 20 and 110 μ g. (55, 56, 59); that of the fetal dog is 5 μ g., as base (36).

The first evidence for the release of a vasodilator and smooth muscle stimulating substance into the circulation from the liver during the anaphylactic shock was discovered by Manwaring and his co-workers (95, 96). As early as 1911, Manwaring had suggested such a mechanism. Their experiments demonstrated that the active principle was released into the circulation in concentrations sufficient to produce effects on distant organs. Further positive results were obtained by Simonds & Brandes (116). Later it was shown (35, 63, 64) that the lymph and blood collected from the liver during shock contained a substance resembling histamine in its physicochemical properties and its pharmacological effects. The identity with histamine was strengthened by the fact that the substance was inactivated by histaminase (37). The degree of shock was proportional to the amount of histamine released. Secretion of acid gastric juice obtained during shock has also been attributed to liberated histamine (120). More recently Code (26) has found that there is a sharp rise in "blood histamine," sometimes rising from less than 0.01 to over 1.0 μ g. per cc. of blood within the first ten minutes after the injection of the antigen and then rapidly fall-

ing off, but not returning to its former level until two to three hours later. The normal so-called blood histamine is mainly derived from the leucocytes (25, 28), but the increase during shock resulted from the appearance of free histamine in the plasma. "The duration of the lowered blood pressure was related to the concentration of histamine attained in the blood and the time required for the excess histamine to disappear." Histamine injected intravenously disappeared from the blood more quickly than it took for the increased blood histamine in anaphylactic shock to return to its former level. This suggests an initial considerable but short release, followed by a long lasting slow output which corresponds to the release observed from isolated guinea pig's tissue. Some of the cellular histamine of the blood appears to be released into the plasma when antigen is added to blood of sensitised dogs (81).

Guinea pigs.—The dominant symptom of acute anaphylaxis is broncho-constriction and the lung may be regarded as the shock organ. Its histamine equivalent varies between 4 and 94 μ g. per gm. of tissue (34, 53, 59, 94). These individual variations have to be taken into account when changes in the lung histamine are evaluated. The great differences said to occur at different periods of sensitization (Hosoya, Watanabe) and the increase described after injections of histidine (19) could be attributed to these individual differences (13, 34, 94).

Bartosch, Feldberg & Nagel (12, 13) perfused the lungs of sensitized guinea pigs. After the injection of the antigen, a substance appeared in the venous perfusate resembling histamine in its physicochemical properties and pharmacological effects. A few similar observations have been made by Spinelli (118). The results have been confirmed and extended (33, 34, 127) and it was shown that the active principle was inactivated by histaminase. The histamine released from the lungs amounted to between 0.17 and 12.8 μ g. Similar results have been obtained from passively sensitized animals (112). There was a parallelism between the degree of bronchoconstriction and the amounts of histamine released (12, 112) which was particularly striking in the experiments on passive anaphylaxis. Ungar & Parrot (125) suspended the isolated intestine of a normal guinea pig in warm Tyrode solution containing lung fragments from a guinea pig sensitized to horse serum. The addition of serum in amounts, which in themselves had an insignificant stimulating effect, produced, after a short latency, a strong con-

traction which was attributed to the release of histamine from the lung fragments. A modification of this method has been used independently by Schild (114, 115). Small samples of tissue were suspended in warm Locke solution; the antigen, egg albumen, was added to the solution and left in contact with it for ten minutes; the solution was then assayed for histamine. The release of histamine per gram of tissue varied between 0.5 and over $3\mu\text{g}$. (expressed as base).

The following experiments demonstrate that the histamine is released from the lungs and not formed either from the antigen or from the tissue. The histamine content of successive samples of venous perfusate collected from perfused shocked lungs did not run parallel with the egg albumen content of the samples (13). The appearance of histamine in the venous perfusate was associated with a corresponding decrease in the histamine content of the tissue as shown by experiments in which one lung was subjected to shock, while the other was used as a control (11). In two perfused lungs, Schild (113) produced shock with 1 and $10\mu\text{g}$. egg albumen respectively. If all of the albumen histidine had been decarboxylated, it would not have accounted for more than 12 and 16 per cent, respectively, of the histamine found in the perfusate, the maximum histidine content of egg albumen being 2.5 per cent (23). There is no variation in the histidine decarboxylating power of the guinea pig's kidney during sensitization or shock (100).

Schild (114, 115), using the method described, estimated the release of histamine from different tissues. From the aorta it amounted sometimes to over $6\mu\text{g}$. per gm. (average, $3.8\mu\text{g}$. per gm.). The release was depressed at low temperatures and occurred apparently in two stages, an initial reaction during the first few seconds contact with the antigen, requiring higher temperatures, and perhaps representing the actual release from the cells, followed by a second slower phase which could proceed at lower temperatures and might represent the diffusion of the released histamine from extracellular spaces into the bath fluid. From other organs the average release in μg . per gm. of tissue was about 1.6 for the uterus, 0.6 for the seminal vesicles, 0.5 for the esophagus, 0.4 for the heart, 0.3 for the bladder and $0.2\mu\text{g}$. for the skin. The esophagus was the only part of the alimentary tract liberating considerable amounts of histamine, the release from other parts being nearly always below threshold, i.e., below $0.05\mu\text{g}$. per gm. Differ-

ences in the histamine content of the tissues were not responsible for these variations, since the uterus and ileum yielded approximately identical amounts of histamine on extraction.

The "blood histamine" of guinea pigs was found to be increased five to ten times during anaphylactic shock (26), probably due to the appearance of free histamine in the plasma, as in the corresponding experiments on dogs. This question, however, has not been examined in the guinea pig. The histamine may have been released from the lungs or other organs or even from the leucocytes, since a release of cellular blood histamine into plasma has been observed (81) when antigen is added *in vitro* to blood of sensitized guinea pigs.

Rabbits.—The "blood histamine" decreased during anaphylactic shock (108), this fact probably indicating a decrease in leucocytes in the circulating blood (51). The plasma histamine usually did not increase, although occasionally a rise took place. The only really positive evidence in sensitized rabbits concerns the release into the plasma of a considerable fraction of the cellular histamine from the blood when antigen was added to it (41, 81).

Other animals.—No increase in "blood histamine" occurred during the anaphylactic shock in the horse, sheep, goat, and calf. Code & Hester (27) observed in fact a decrease, which, in the horse where it was examined, was associated with and probably explained by the disappearance from the blood of the white blood cells.

Factors influencing the release.—Mention has been made of the effect of temperature. Epinephrine was found to decrease the release of histamine from perfused shocked lungs of guinea pigs (112). Diethyl ether had a similar effect, as shown in perfusion experiments and on suspended samples of sensitized tissue (82).

The individual variations in the histamine content of the tissues have hitherto not been related to the strength of anaphylactic reaction and to the variations in the release of histamine. In the experiments on passive sensitization, release and response depended on the degree of the condition.

RESPONSES OF SMOOTH MUSCLE PREPARATIONS TO HISTAMINE AND ANTIGEN

There are responses, such as that of the rat's uterus and the guinea pig's uterus poisoned by histamine, which are difficult to

explain on the assumption that histamine is the sole factor responsible for all anaphylactic contractions, but these instances do not invalidate the evidence that release of histamine is mainly responsible for the normal anaphylactic contractions of those muscles which are sensitive to histamine. The guinea pig's uterus, for instance, contains between 9 and 20 μ g. of histamine per gm. of tissue, 0.6 to 2 μ g. of which are given off if samples of the tissue are brought into a solution containing the antigen. A solution containing less than 0.1 μ g. per cc. is usually sufficient to contract the muscle. The guinea pig's small intestine has about the same sensitivity as the uterus and yields about the same histamine equivalent on extraction, but Schild (115) could detect no histamine if samples of tissue from a sensitized animal were brought into a solution containing the antigen. We do not know whether for anatomical or other reasons the released histamine is unable to diffuse into the bath fluid, or if there is a great quantitative difference in the actual release from these two tissues in which the anaphylactic reactions appear to be so similar.

There are a number of observations which more or less support the histamine theory of anaphylaxis although they do not explain the differences mentioned. Formaldehyde (87, 88), urethane, chloralose (45), the phenol ether thymooxyethyldiethylamine (20, 21, 109), and pretreatment with histamine (47) and arginine (3) render the guinea pig's uterus and intestine more or less insensitive to histamine and to antigen. However, the fact that desensitization takes place indicates that the antigen-antibody reaction has occurred. The value of these observations for the histamine theory varies. The effect of formaldehyde consists in a chemical reaction with the amino group of the histamine (87). This explanation gives these experiments a high value for specificity. Urethane and chloralose appear to have a general depressant action on the excitability of smooth muscles and no claim for the specificity of the effect has been made (46). Nor is there evidence that pre-treatment of a guinea pig with histamine specifically depresses the histamine response of a smooth muscle preparation taken from such an animal. Even the phenol ether for which a specific antagonism to the histamine response has been claimed (109) depresses the effects of other stimulating substances (3). According to Ackermann & Wasmuth the histamine-arginine antagonism is alone specific. However, there has been no investigation of whether arginine in-

fluences the reactions of those substances which may be additional intermediaries in anaphylaxis (see below).

Discrepancies in histamine action.—Desensitization of a muscle sensitized to antigen does not influence its response to histamine. This is no argument against the participation of histamine in anaphylaxis since desensitization would affect the mechanism of liberation. Against the histamine theory has been cited the anaphylactic contraction of the rat's isolated uterus (85), which is usually relaxed by histamine. The weak stimulating effects observed on this muscle *in situ* on intravenous injections of antigen as well as histamine (124) may be indirect nervous actions and cannot explain the discrepancy existing for the isolated uterus. It is also not sufficiently explained by the facts that minute amounts of histamine (1, 80) or extremely strong concentrations (101) may contract the isolated uterus and that rather large amounts of antigen are necessary to produce an anaphylactic contraction, which even then is not very powerful. Another discrepancy is provided by the guinea pig's uterus poisoned with strong concentrations of histamine. It responds to further histamine with relaxation but to the antigen with contraction (113). Schild concluded "that either the histamine released from the cells has a different action from that of histamine applied to the cell outer surface or it plays only a secondary part in anaphylactic shock." There are other smooth muscle preparations, such as the rabbit's intestine which give anaphylactic contractions and which, in comparison with the guinea pig's uterus or intestine, are rather insensitive to histamine.

The possibility that there is a factor other than release of histamine which may contribute in anaphylactic contractions has been approached from observations with snake venoms. In 1929 Kellaway (84) described the close resemblance of the effects of different snake venoms on the guinea pig's uterus to those of the anaphylactic response and raised the question of whether the same stimulant substance might be produced in the tissues in both conditions. He discarded histamine since the venoms contracted the rat's uterus. Later other venoms and their effects on other smooth muscles were examined and all those discrepancies were met with which had been observed between histamine and the anaphylactic response. For instance, cobra venom causes contraction of the rabbit's intestine, the uterus of rats and mice, and that of the guinea pig poisoned with histamine (56, 51).

Snake venoms are enzymes splitting the proteins and the lipins of the tissues. In both processes, in addition to the release of histamine, substances appear to be formed with actions which could explain the differences observed on smooth muscles. The stimulating effect of crystalline trypsin on smooth muscle preparations has been studied by Rocha e Silva (106, 107). The guinea pig's uterus contracted in response to a concentration of one in thirty millions; next in sensitivity came the small intestine of the guinea pig, then that of the cat, rabbit, and rat. The same order of sensitivity holds good for histamine. The intestine of the mouse, which is nearly insensitive to histamine (129), was also found to be refractory to trypsin. This correspondence is suggestive. On the other hand, the rat's uterus is contracted by trypsin. This response may be an effect of peptones, which must be formed at some stage of the trypsin digestion. Peptone contracts the uterus of the rat and that of the guinea pig poisoned by histamine (51, 56), it stimulates the rabbit's intestine, but has no stimulating effect on the intestine of the mouse (51). Release of histamine together with formation of peptones from the tissue proteins thus could account for all the known smooth muscle stimulating effects, not only of trypsin but also of venoms and anaphylaxis.

When cobra venom or other venoms are incubated with a sample of lecithin or egg yolk and then freed from the venom activity by heating or alcoholic extraction, the extracts have not only a lytic but also a smooth muscle stimulating action (56). It was thought at first that both actions were properties of lysolecithin, but, when it was found (52) that the activities could be segregated in different fractions, this view could no longer be held. The muscle stimulating principle has not been identified. Since all samples of lecithin contain impurities, it may not necessarily be a split product of lecithin. It is neither oleic acid nor a simple oleate (52). It cannot be phosphorylaminoethanol, phosphorylcholine, or oleylcholine which have little or no stimulating action on smooth muscles (51). No muscle stimulant (or lytic) substance is formed by the action of cobra venom on crystalline egg albumen (51, 74), sphingomyelin, or phrenosin. It is not identical with von Euler's and Gaddum's P-substance (51). Holden (74) tried without success to purify the active principle but his attempts resulted in almost complete disappearance of pharmacological activity. The active principle is also formed by venoms in the living tissues (52, 55, 56) and

has been termed (86) "slow reacting substance" because it contracts the guinea pig's intestine more slowly than histamine, and after washing out the muscle also relaxes more slowly. Like peptone "slow reacting substance" imitates more closely than histamine the muscle stimulating properties of snake venoms as well as of anaphylaxis. It contracts the rabbit's intestine, the rat's and mouse's uterus, and the guinea pig's uterus poisoned by histamine (51, 52). The intestine of the mouse is not contracted by either this substance or cobra venom (51).

In the case of venoms, the formation of "slow reacting substance" appears to be closely linked with that of lysolecithin, but in other instances the substances may be formed independently of each other. For instance, incubation of plasma or serum at body temperature renders blood more stable to sedimentation, probably due to the formation of lysolecithin by the action of a blood lecithinase (15, 16, 48), but there is no simultaneous production of "slow reacting substance" in this process (49).

Saline extracts of tissues have an action on the isolated guinea pig's jejunum, like that of "slow reacting substance" usually preceded by that of histamine. With extracts from the monkey's liver which contains traces of histamine only, the slow contraction is the sole response (56). There is no evidence that the substance responsible for this effect is identical with the "slow reacting substance." In that case it could be liberated and would not necessarily have to be formed during the antigen-antibody reaction. To test these possibilities Kellaway & Trethewie (86) produced various states of decreased excitability by different means in the muscle of the uterus and jejunum of the guinea pig with the idea of obtaining conditions under which the responses to histamine, to antigen, and to "slow reacting substance" were affected differently. The results were not conclusive and illustrated the difficulties of drawing conclusions from such experiments.

Release of a muscle stimulant substance other than histamine.—In further experiments Kellaway & Trethewie (86) perfused the lungs of sensitized guinea pigs. After the injection of the antigen there appeared in the venous perfusate not only histamine but also a substance causing a slow contraction of the guinea pig's jejunum. They referred to this substance as "slow reacting substance" without claiming to have established its identity with the substance formed by venoms on lipins. It may even have been peptone. The

output of "slow reacting substance" bore no relation to the amount of histamine liberated, the maximum output of which preceded that of "slow reacting substance." Results obtained with tissue extracts suggested to them that the substance was liberated from pre-existing stores. There was an increase in "slow reacting substance" content of fresh extracts from perfused sensitized lungs after arterial injections of the antigen or incubation with it. However, dried extracts, which yielded more of the substance from normal tissue than did fresh extracts, revealed no increase in the content of this substance when antigen had been allowed to act on the sensitized tissue. They concluded "that the anaphylactic contraction of smooth muscle is in part due to liberation of histamine and probably in part also to the "slow reacting substance" which is liberated and not formed in the antigen-antibody reaction." The experiments therefore yielded no evidence in favour of the attractive theory that "slow reacting substance" is formed enzymatically during the antigen-antibody reaction from tissue lipins. Recently Campbell & Nicoll (24) have made an observation probably dealing with the same phenomenon. Lung fragments from sensitized guinea pigs were suspended in Ringer's solution and were found to release, on the addition of antigen, a substance which contracted the uterus of the rat. They discussed the possibility of its being a choline-like substance. It may have been "slow reacting substance" or peptone.

THE ROLE OF HISTAMINE IN THE ANAPHYLACTIC SHOCK OF DIFFERENT ANIMALS

In comparing the effects of injected histamine with anaphylactic reactions several factors have to be taken into account. Injected histamine will flood the whole circulation reaching all the tissues. The chief effect of the released histamine is a local one, acting in the tissue spaces into which it is released. Only part of the histamine will diffuse into the blood stream and produce effects on distant organs. The histamine in the blood gives only an approximate picture of the amounts actually released. The proportion reaching the blood appears to vary greatly with different species, probably according to the histological structure of the tissues affected. Since certain organs are the main site of the antigen-antibody reaction in most animals, the effects on these organs will dominate the symptomatology. Accordingly, differences between the symptomatology of

the response to injected histamine and anaphylaxis do not necessarily disprove the histamine theory. On the other hand, the importance of similarities of symptoms also should not be overstressed, since different mechanisms may produce similar symptoms. We have further to realize that the histamine theory does not pretend and never pretended to reduce all manifestations of the antigen-antibody reaction to histamine effects. The injury is, as Dale put it, a far more severe and disorganizing one than would be necessary to produce a physiological release of histamine and it involves other and more direct results. The role of histamine, therefore, may vary greatly for different symptoms, and such statements as that it is of primary or secondary importance are of little value. The triple response of the urticaria rash in allergic patients may be accounted for by release of histamine, whereas the Arthus phenomenon, another skin reaction of anaphylaxis, appears to be a manifestation of the injury without participation of histamine. The more chronic effects are most likely a result of the injury itself, whereas the release of histamine is a factor to be reckoned with in acute manifestations.

In the acute anaphylactic shock of dogs the released histamine reaches the circulation in amounts sufficient to explain most of the extrahepatic histamine-like effects on the circulation and smooth muscles and there is a striking parallelism between the degree and duration of shock and of release of histamine (26, 35). In the liver the effect of the released histamine on the hepatic veins may accentuate the tremendous swelling and increase in lymph flow, both symptoms, as well as the release of heparin, resulting from the injury itself, possibly being symptoms of intracellular peptone formation.

In rabbits the acute shock results in a steep fall of arterial blood pressure, whereas histamine usually causes a rise. The early experiments of Airila, Coca, and others have shown that the anaphylactic depression results from constriction of the pulmonary arteries. The effect has been demonstrated on isolated strips of the artery (70) and by microscopic study of sections of lungs suspended in Ringer's solution (66). The main release of histamine therefore must occur around the muscular wall of the pulmonary arteries. Thus its local action at the site of liberation determines the circulatory event. The pressor effect of injected histamine results from constriction of the systemic vessels, but constriction of the pulmonary artery occurs and may even become the determining factor,

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as in anaphylactic shock. Dale & Laidlaw had already pointed out that it is necessary to depress the sensitivity of the pulmonary vessels to histamine in order to obtain the sharp rise in systemic pressure. This is often followed by a slow fall with incomplete recovery for several minutes, and, following repeated injections of histamine, the depressant aftereffect may become more and more evident, so that a low level of arterial blood pressure results (107). On the other hand, anaphylactic constriction of systemic vessels has been observed in the rabbit's ear in perfusion experiments (62, 65) and in microscopical studies of the vessels under natural conditions of circulation (2). In addition, repeated short-lasting obstructions of the pulmonary artery change the response to antigen which produces then, like histamine, a rise in systemic blood pressure (107). The treatment probably weakened the response of the lung vessels so that the effect on the systemic vessels became predominant.

Histamine has also a vasodilator action in rabbits. Small doses injected intravenously contract the central artery but dilate the capillaries and venules of the ear (50). According to Grant, the dilation extends even to the small arteries (68). No anaphylactic vasodilation occurs in the ear, but the constriction affects the arterial side only, leaving capillaries and venules unaffected (2). These apparently are not involved in the antigen-antibody reaction and the fact that they dilate if histamine reaches them from their lumen does not create a difficulty with regard to the histamine theory. There remains the dilation of the smaller arteries. Possibly this discrepancy would disappear if the effect of histamine and antigen on the vascular tree were compared under similar conditions by one and the same worker. The accumulation of leucocytes which occurs at the vascular wall during the anaphylactic contraction (2) must be regarded as a response to the injury itself.

In guinea pigs the acute anaphylactic shock with its fatal bronchoconstriction can be imitated by histamine and may be wholly the result of its liberation. Histamine does not imitate the striking eosinophilia and the characteristic chronic histological changes in the lungs (77). These probably are reactions to the direct effects of the injury. It would be interesting to know if peptone would reproduce these signs.

Urethane (46), pre-treatment with histamine (9, 47, 78, 117), histaminase (79), and the phenol ether thymoxyethyl-diethyla-

mine (20, 21, 109) have been found to protect the guinea pig against a normally fatal dose of histamine or antigen although desensitization occurred in the latter case. The protective action of histaminase against histamine or anaphylactic shock is not an unanimous finding (29). The effect of urethane cannot be regarded as specific and the claim (109) that the protective action of the phenol ether is specific for histamine has also been denied (3). Rosenthal & Brown (109) used this ether to differentiate between histamine symptoms and those due to primary cell injury. The ether prevented the acute symptoms of a usually fatal dose of antigen, but did not prevent the signs of malaise and prostration which could last for hours and were attributed to the tissue injury itself. The importance of these experiments is lessened by the fact that when antigen was injected greatly in excess of the normal fatal dose, it produced typical shock.

The anaphylactic shock in rats and mice has been used as an argument against the histamine theory. In order to produce a fatal histamine shock in these animals several milligrams have to be injected intravenously. It is therefore unlikely that effective amounts are released in anaphylaxis, the histamine content of the different tissues being of the same order in rats and in guinea pigs (97). On the other hand, mice, and to an even greater degree, rats, are also peculiarly tolerant to antigen. In rats the symptoms are extremely mild and evanescent. In fact, Went & Martin (130) observed no signs of anaphylactic bronchoconstriction in perfused lungs and no blood pressure effects. After removal of the adrenals, when the animals become markedly more sensitive to histamine, they also become susceptible to anaphylaxis, the predominant reaction in both conditions being a fall in arterial blood pressure. (124). The literature on this subject has been reviewed by Went & Martin (130). In mice the anaphylactic symptoms are again always less dramatic and less acute than in guinea pigs, resembling the protracted shock in the guinea pig as it occurs after a previous injection of thymooxyethyl-diethylamine, which antagonizes the histamine effects. It is possible therefore that the mild anaphylactic reactions in mice and rats are mainly the direct outcome of the "cell injury," that release of histamine only slightly accentuates the reactions, and that their insensitivity to histamine determines the tolerance to anaphylactic shock.

ALLERGIC CONDITIONS IN HUMAN BEINGS

Anaphylaxis is only one instance of allergy, and the hypersensitivity of some persons to specific allergens is generally assumed to be a similar immunobiological phenomenon. There existed some doubt on this point because animals are easily desensitized by reinjection of the antigen, if they survive the attack, whereas allergic attacks, such as asthma or hay fever, usually leave behind no real condition of desensitization. Under similar experimental conditions, however, this difference disappears. In guinea pigs sensitized to protein, repeated and typical asthmatic attacks can be elicited without producing desensitization, when the protein is given by inhalation in a fine spray (77). These findings may perhaps warrant attempting to desensitize allergic patients by intravenous injection of the specific allergen in a dose sufficient to produce an attack, which may be alleviated by epinephrine or ephedrine without preventing the development of desensitization.

Evidence for the release of histamine in allergic conditions is confined to experiments on the human skin. It is mainly indirect but nevertheless conclusive and rests upon the fact that the uncomplicated triple response to a variety of stimuli is the result of liberation of histamine from injured or irritated epithelial cells. Some people display extreme susceptibility to certain protoplasmic substances (such as extracts of fishmeat, or pollen) which may induce attacks of asthma, urticaria, and eventually signs of collapse. From examination of their skin reactions, it could be concluded that the substances act as cytotoxic stimuli liberating histamine. The literature on this point is reviewed by Lewis (92). Related to these phenomena are certain instances of increased susceptibility of the skin to irritant substances, some of simple chemical constitution. The susceptibility to acetylcholine has a special physiological interest. People, mainly females, who display urticaria on emotion, exercise, or warming of the body were found to be susceptible to acetylcholine which acted as a cytotoxic stimulus releasing histamine and producing the triple response (69). In the attacks, the acetylcholine is released from endings of cholinergic nerves in the skin, but the nerve fibers responsible have not been traced. Atropine prevented the effect. A similar mechanism may be involved in some cases of nervous or psychic asthma.

Systemic reactions involving the blood pressure, heart rate, and gastric secretion which could be attributed to diffusion of suf-

ficient histamine into the blood stream have been described in cases of urticaria factitia [the literature on this matter being cited by Lewis (92)] and more recently in cases of cold allergy (10, 75).

Apart from being liberated during an antigen-antibody reaction, histamine may be of importance for symptoms sometimes difficult to distinguish from genuine allergic reactions. The "cotton dust asthma" observed in card room workers may not be a reaction against the dust particles as allergens but may in part at least result from histamine present in the dust and inhaled with it (72, 93, 102). Crystalline histamine salts could be prepared from the dust (72), but dust from esparto grass and coir was practically free from it and workers exposed to this kind of dust did not complain of respiratory troubles as did the cotton operators (93). The "blood histamine" of card room workers suffering from respiratory troubles was found to be on the average on a higher level, and single individuals showed greater variations on repeated examination than was found in control groups (72).

Histamine may also be derived from bacterial decarboxylation of histidine in the lungs. Asthmatic sputa may contain a histamine-like substance and also bacteria which apparently are able to produce histamine (90). That there is at present no evidence that absorption of histamine from the intestinal tract may lead to allergic-like manifestations in human beings is the conclusion from study of the earlier literature by Feldberg & Schilf (59). Founder, an equine disease induced by food, must probably be explained along these lines (5). It occurred only in animals which had a rich flora of histidine-decarboxylating bacteria and which had recently received fodder rich in histidine. Founder, with its typical affections of the joints, could be induced in normal horses by feeding a diet rich in histidine or pure histidine, and introducing simultaneously by stomach tube a flora of histidine-decarboxylating bacteria. In animals, whose upper intestinal tract contained such a flora, food rich in histidine was sufficient in itself to elicit an attack. Åkerblom (6) discusses the possibility of histamine absorption as a cause of rheumatic manifestations in human beings.

Histamine and histaminase treatment.—In order to reduce the sensitivity of the reactive cells to released histamine, injections of histamine as treatment against cold allergy, allergic skin diseases, migraine, asthma, and hay fever have been used with success (8, 22, 42, 76, 119). The histamine theory has further led to the

examination of the histaminase factor in allergic conditions and their treatment. According to Albus (7), the histaminase activity of the blood is lowered in cases of latent allergy. Marked relief is said to be obtained by histaminase in cases of cold allergy (10, 110), serum sickness (61), allergic insulin reactions (111), gastrointestinal allergy (4), numerous allergic skin reactions (60, 67, 71, 91, 99, 126) and hay fever (44). But the results have not been uniformly confirmed and reports of negative results have been made for cold allergy (89), hay fever (83, 98), asthma and allergic skin reactions (98).

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PHYSIOLOGICAL LABORATORY
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EXERCISE

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INTRODUCTION

The organism's capacity for muscular work is determined by many factors, among them constitution, sex, and age. In turn the performance of work changes the entire individual. Some changes are temporary adjustments which sustain work and which fail in fatigue. Others are the more lasting changes which mark the trained state and condition subsequent performance. Some may serve to predict performance. The endocrines, in part, shape the nature of performance and in turn do not escape modification. At times muscular performance is suspected of injuring the body. There is also some evidence of therapeutic value. It assumes forms ranging from complete muscular relaxation, through simple postural defiance of gravity to the most strenuous and complex body movements in individual and cooperative work and sport forms, some of which are beginning to succumb to analysis.

CONSTITUTION, SEX, AND AGE AS FACTORS WHICH DETERMINE PERFORMANCE

From a comparison of forty-seven anthropometric data secured on fifty-one negro and fifty-one white American college students, it appears that the negro has body proportions such as to give him, theoretically, a slight kinesiological advantage in jumping, throwing, and speed events, whereas for events of endurance he is somewhat handicapped (108). The grip strength of Mongolian students in Cha-lan-tun is markedly inferior to that of Japanese (166). Among thirty-four Harvard students and members of the faculty, those possessing flatter chests, longer torsos, shorter extremities, and narrower hips (linear types) were found to have a higher basal metabolism and a lower mechanical efficiency in moderately strenuous work, but greater endurance in exhaustive work than the opposite or lateral types (163). Nervous types of individuals exhibited greater variations in blood flow through muscles than did phlegmatic types (61).

Again certain German clinicians lament strenuous athletic competition, and especially masculine sports performed during the period of early adolescence, as responsible for damage to female sex functions (19, 174); but another (156) is satisfied that the masculine type found among female athletes is the cause of her ability and not the result of her indulgence in events designed for men.

The muscles of rats attained their full strength at ninety days of age (87). Observations on eighty girls and eighty-five boys through four years of adolescence in California indicates that the performance ability of boys increases throughout this period, but girls reach their maximum at about fourteen years in dashes, broad jump, target, and distance throws with a tendency to decline thereafter. In events featuring co-ordination, however, girls also continue to improve (38). One Japanese boy observed at the age of fifteen and again at sixteen exhibited a change in mechanical efficiency from 4.5 per cent to 6.5 per cent in weight lifting. Over the same period his optimal load shifted from 8 kg. to 18 kg. (172). Boys aged ten to fourteen years were found to suffer no measurable ill effects from bicycle rides of 72 km. in one day. They performed three minutes of energetic work with a 10 kg. load as well after riding 72 km. as on control days. Respiratory analyses showed rapid return to normal in all functions. Exceptionally low oxygen debts (maximum 3.8 l.) were found in all boys who rode a loaded ergometer as long as possible. A predisposition to avoid high oxygen debts was apparent, but unexplained (89, 176). The recent ten-second rule without center jump is believed to have made the game of basket ball too strenuous for junior high school boys (18); the data are not convincing.

POSTURE

Adjustments to gravity may be required of the circulatory system with or without the aid of contractions in antigravity muscles.

The cost of vertical posture varies from 2.58 extra kilocalories per hour for suspension in water and 5.42 for the mechanically supported position on a tilting table to 19.23 kilocalories per hour for ordinary standing with swaying. Since the fatigue of motionless posture cannot be due to metabolite accumulation at such low costs, it is attributed to a cerebral anemia due to the absence of the peripheral pumping effect of postural sway (51). The minute volume is not reduced in changing from lying to standing posture as

claimed by others. The human organism is able to maintain a constant cardiac output in any self-sustained posture. This does not include passive tilting (43). A larger heart and a shift of the region of greatest pulsation from the apex of the heart to its base were found when kymographic x-ray records of men standing on their heads were compared with those taken in normal standing (60). On a tilting board, heart rates up to one hundred accompanied the passively held head-up position and rates as low as thirty-five accompanied the head-down position (7). "Shortening" the blood columns by inflating cuffs (300 mm. Hg) about the thighs reduced the magnitude of pulse rate alterations. During the reactive hyperemia following sudden cuff removal even greater increases in pulse rate were observed in the vertical position. These were paralleled by a drop in blood pressure (8). The performance of arm work against an elastic resistance during tilting experiments did not alter the hydrostatic effects (10). Passive upright posture of 45° could be maintained on a tilting board for an hour in the face of diminished heart output without a drop in blood pressure. A diminished diuresis following water by mouth, a slight proteinuria, and a simultaneous increase in the protein content of blood serum are believed to reflect impairment of kidney secretion and intestinal absorption ascribable to the visceral vasoconstriction which compensates for the diminished minute volume (9). Under similar experimental conditions a falling skin temperature in the extremities indicates constriction of the peripheral blood vessels. This adjustment though helping to maintain blood pressure modifies the factors influencing heat loss. The consequent rise in rectal temperature (0.5°C.) may be a factor in the ultimate collapse (124). The speed of circulation to the feet is decreased in standing when estimated by a method which depends on the reporting of a sensation of warmth felt in the skin. This change is also attributed to vasoconstriction (94). Three patients are described who apparently lacked the circulatory adjustments necessary to maintain erect posture. Entirely normal while sitting or reclining these individuals suffered marked hypotension, tachycardia, and extreme exhaustion on assuming the erect posture and walking a few steps (100). The effect of centrifugal force on the blood columns of pilots executing sharp turns in flying and power diving is described. It appears to be an accentuated instance of the posture effect. Under conditions imposing a force five times gravity toward the feet,

vision is lost while a force six to nine times gravity results in fainting. Pilots vary in their ability to withstand gravity. Tensing the muscles of legs and abdomen, or shifting the body axis appropriately may also prevent complete cerebral anemia. A lower tolerance (maximum 4.5 g.) is found to such turns as outside loops which drive blood into the head (140).

The vital capacity is from 223 to 500 cc. less in the recumbent position than in standing. This reduction is due to the larger amount of blood in the chest cavity while reclining (11, 31, 102). The difference between the vital capacity of lying and standing is reduced when cuffs are inflated around the thighs to prevent blood entering the legs (11, 31). Apparently blood running into the leg vessels makes room for more air to enter the lungs. X-ray observations and estimations of residual air indicate that whereas this reduction in vital capacity in recumbency is not due to changes in thoracic measurements, the normally higher breathing level, i.e., larger functional residual air in this position, is occasioned by a purely mechanical upward displacement of the diaphragm (102).

ADJUSTMENTS DURING AND IMMEDIATELY FOLLOWING EXERCISE

In respiratory mechanisms.—After a single period of two to three hours of forced swimming the lungs of rats showed an increase in weight due to infiltration of fluid and to exudations of albuminous fluid. In restricted areas there was a stretching of alveoli with occasional hemorrhages into the alveoli (127). This may account for the "taste of blood" sensation sometimes noted in strenuous exertion. In more extensive exertion the same paper reports that alveolar septa were stretched to the point of complete compression of the contained lung vessels thus accounting for the blockage of pulmonary circulation and the increased burden on the right heart. Increased respiratory minute volume in the recipient following transfusion of blood from a strenuously exercising donor leads to postulation of a purely humoral stimulus for the increased respiration of exercise (86). The conclusion is not as startling as the experimental procedure. Breathing movements while swimming require from 2.77 to 5.97 kg. m. of extra energy because of the water pressure against the submerged chest and the more rapid inhalations demanded in a perfected stroke (79). Vital capacity and residual air were found to be unchanged during and after a

short period of strenuous work. Mean capacity (residual air plus one half tidal air) fluctuated irregularly. The contrary observations of others may be due to their not having made all determinations in the same posture (6, 63).

In the circulation.—Following the onset of "second wind," however, there is a marked reduction in vital capacity. This and a simultaneous increase in oxygen intake and other logically related changes are taken as evidence that increased venous return and an increased cardiac output are the main factors in initiating "second wind" (159). Frequent liver enlargement after long distance running was found in poorly conditioned athletes (155). Increased diastolic pressures during and immediately after weight lifting are attributed to constriction of the arterioles with the assistance of contracting muscles. There is postulated an "arteriolar-capillary" reflex which co-ordinates arterioles and capillaries reciprocally and does not permit dilation of both simultaneously. Its failure would incapacitate for work (149). Blood pressures taken by (a) auscultatory, (b) tonoscillographic and (c) "energometer kurve" methods before and after work may vary a little, but the methods are equally accurate for following the course of blood pressures in recovery (37). Direct thermostromuhr determinations on dogs running three miles per hour on a level course or on 15° inclines indicate a markedly increased blood flow in the common iliac artery but no compensatory reduction in the renal or superior mesenteric arteries, or in the splenic artery or vein as formerly deduced. At times there was, in fact, a definite increase. Any vasoconstriction in these regions is evidently counterbalanced by the increased blood pressure (53). Cardiac acceleration up to 286 beats per minute was found in dogs during excitement and exercise one year after complete sympathectomy. The accelerations which began to manifest themselves fourteen days after the operation were much reduced subsequent to double vagotomy (20). Almost identical findings are reported for operated cats although the cat's performance suffers more than that of the dog. It is somewhat improved by injections of epinephrine (58). Over a period of ten weeks a case of acute cardiac dilatation brought on by athletic exertion showed complete laboratory, clinical, and athletic field recovery (84).

In blood and tissue fluids.—Reductions in blood sugar due to long, strenuous exercise were observed in man (46) and in the dog

(152). Others (115) who recognize cyclic changes in blood sugar based on an autorhythm in liver function find that the regulation of blood sugar in man is sufficient to maintain the blood sugar level in the face of work performed in various phases of liver activity. In rats a secondary rise in blood sugar was found to follow upon a reduction due to exercise (164). An increase in serum chloride during exercise is reported for the dog (116) and for the rat (164) to persist for several days following exercise. Erythrocyte and muscle chlorides were increased in a heart-lung-gastrocnemius preparation receiving indirect stimulation (180). The livers of cats lost whereas their muscles gained chloride and water during muscular activity (39). No serious chloride shortage was found in miners even though they secreted 3 to 6 kg. of sweat in a shift. This is attributed to the small amount of water consumed and to additional chloride taken with a light lunch. There appears to be no need for salting the drinking water under these conditions (97). Whether blood chloride is a dependable measure for the adequacy of body chloride is made questionable by the exercise hyperchloremias reported above. Potassium is lost by erythrocytes in fifty-five seconds of strenuous work (83), but gained in longer work (180), lost from serum of running dogs (116), but gained by serum of man in short strenuous exercise (83). During electrical stimulation muscles lose potassium at a rate proportional to the flow of blood through them (180). The liver stores this potassium (39) to return it at least in part to the muscle during recovery (39, 180). During activity muscles gain water at the expense of the skin (39).

In oxygen consumption, in the flow of blood, and in the use of fuel substances.—The rate of oxygen consumption during work of varying intensity was unchanged by reduction in oxygen content of the respired air. Such reductions did not interfere with performance until the arterial saturation levels were inadequate to prevent accumulation of lactic acid in the face of mounting tasks (88). Cardiac patients obey the same rules (48). Muscle lactate varies inversely with arterial saturation (142). The length of time that maximal static contractions can be held in man and the subsequent recovery time are unaffected by reductions in atmospheric oxygen; but the longer held submaximal static contractions are shortened in a degree proportional to the extent that they depend on an oxygen supply through the partly unblocked circulation (117). The length of time that static contractions could be main-

tained in weight lifting (120) and in foot extensions (13) was found to be related to the amount of blood flow cut off by the contracting muscles; light loads accompanied by a marked hyperemia could be held indefinitely, but maximum loads as little as three seconds. The holding time for maximum loads is unaffected by complete obliteration of blood supply with a pneumatic cuff (13). Thus endurance in static work is also a function of oxygen supply and metabolite removal. The much higher oxygen consumptions for tetanus in acute experiments on dogs (143) than in man (120), i.e., 38 cc. per kg. per sq. cm. per min. for the dog and only 3 cc. for man, and the difference in blood flow during maximum tetanus, which for the dog was found to be 6.7 to 9.2 times resting (91) while for man it was zero, during maximal contractions (13, 120) lead Müller to conclude that animal experiments of this kind are still too unphysiological to render conclusions applicable to man. Probably faradic stimulation in the dog involves continuously the same limited number of motor units, leaving others inactive, whereas maximal exertion in man involves more nearly all of them and submaximal contractions depend on rotation. A maximal oxygen debt of 1.7 cc. per gm. of muscle was calculated for man (120). The superior circulation during rhythmic contractions is capable in man of indefinitely supplying a muscle with 200 times its resting oxygen requirement, whereas a static contraction requiring the same amount of extra oxygen charges 78 per cent of its cost against the oxygen debt (120). Oxygen consumption increases even in the first second of a tetanic contraction in the dog (143). In acute experiments on the dog blood lactic acid rises immediately with slight work and continues high for eight to forty minutes after heavy work (92). The post-exercise oxygen consumption is divisible into a comparatively short phase which may be reduced by factors improving circulation during exercise and a second phase of long duration which though related to the intensity of the preceding work period and to changes in muscle condition cannot be altered by capillary dilatory agents and is unimportant to muscle energetics (91, 143).

Intensive metabolic studies on man indicate that carbohydrates are essential, not preferred, nor ever the exclusive fuel for muscle. The amounts used are related to the work level and their depletion results in exhaustion. Mechanical efficiency was found to be unaltered with changes in R. Q. except at very low levels of

R. Q. The loss in efficiency when observed was ascribed not to a modified combustion mixture but to faulty co-ordination occasioned by pains and nervous disturbances (104). Even after fasting and phlorization the stimulated gastrocnemii of cats showed no appreciable change in fat content whereas carbohydrate stores were much reduced. If fats are used as fuel in muscles, it must be by some indirect route (42). The increase in ketone bodies in the blood of untrained workers which reached its maximum several hours after work was suppressed by dextrose given during work and by mobilization of extrahepatic carbohydrate stores with adrenocortical extract, but increased simultaneously with sugar mobilized from the liver by epinephrine. The latter observation favors a liver origin for exercise ketonemia (179).

A delay in the onset of cataract and the otherwise normal conditions to be observed in rats that are exercised while being fed galactose as a sole source of carbohydrate suggests that this sugar is made available directly or indirectly for muscular activity (113).

No signs of increased protein metabolism were found during strenuous work in two men. Urinary nitrogen, creatine, and creatinine in half-hour samples remained unchanged with the onset of heavy work and dropped gradually through work periods lasting sometimes eleven hours (105). No increase was found in blood creatine after severe exercise in 90 per cent of normal subjects (129). The physiological creatinuria in children on a creatine-free diet could not be modified by dextrose or insulin injections but was completely absent for several days following one hour of exhausting work. The partial retention of creatine fed in the post-exercise period was further evidence of greater creatine utilization following work (145). Men students observed during strenuous physical training on high carbohydrate diets exhibited unusually high urinary creatine and creatinine unrelated to increasing physical fitness. The creatinuria was found to be associated with the high carbohydrate diet. Evidence also was found to support the view that the creatinine of urine is an index to muscle mass (57).

That mild exercise is more effective than bed rest or diathermy in hastening the disappearance of blood lactates during recovery was verified (173). The increase in muscle cystine-cysteine which follows activity is derived chiefly from the muscle itself (70). Sodium azide (Na N_3) possesses the property of inhibiting the activity oxygen consumption whereas it leaves undisturbed the resting

oxygen consumption of frog muscles. The presence of two distinct enzyme systems appears to be the best explanation for the phenomenon (168).

In kidney function.—That albuminuria is physiological after strenuous exertion is confirmed (41, 45, 157). Albuminuria appears after intensive top speed work but not after distance running. Fifteen grams of sodium bicarbonate were sufficient to alkalinize the urine and to prevent exercise albuminuria (45). Exercise albuminuria is less common in the well trained and is reduced by sugar given in lemon juice (157). The alkalinizing effect of the lemon juice is not considered. All of this supports earlier observations. Glycosuria after exercise is uncommon. Increases in red and white blood cells and several types of casts are the rule (41, 157). The excretion of inorganic phosphate is reduced while that of organic phosphate remains unaltered by prolonged strenuous marching (40).

In the nervous system and sense organs.—Stool-stepping followed by push-ups to fatigue was without effect on the response time of simple body movements but a period of basketball or fencing practice significantly shortened response times particularly in movements involving speed and accuracy (34). Blindfolded skiers sliding down a moderate incline changed their direction in response to tonus alterations initiated by head movements. Head bending caused deviations to the same side, whereas head turns resulted in turning their course to the opposite side (15). Football and basketball contests had no significant effect on vision in thirty-six athletes (175). A reflex inhibition of the nerve impulses to a muscle follows a sudden reduction in its load. The sense organs which are activated by a sudden reduction in tension are believed to be in the points of attachment of muscles (167).

THE NATURE AND ESTIMATION OF FATIGUE

A critique of the fatigue problem recognizes depletion of fuel sources as a possible explanation of exhaustion but considers transmineralization and shifting of ions as more important causes of fatigue; especially since these are paralleled by changes in membrane potentials. Beyond these electrophysicochemical phenomena there is postulated a still unknown recovery factor, *Lebenskraft*, which operates to recharge the electric potentials of cells and fluids (121). In a recovery time that is too short to be explained in terms

of metabolite removal and in the fact that a muscle with tied-off circulation recovers from maximal static contractions more quickly than a freely circulated one, there is found reason to seek a cause for fatigue and recovery unrelated to the creation and payment of an oxygen debt (119). The pH of *press juice* from fatigued frog muscles was found in twenty-five tests to average 6.56 as compared with 7.07 for resting muscle (151).

A decrease in skin resistance similar to that which accompanies affective states was found to be invariably associated also with physical and mental fatigue (150). More rapid sedimentation of erythrocytes is reported as a sign of fatigue (121) but is also denied (25). After fatiguing work, urine gave a more pronounced Donaggio reaction than before work; single determinations are meaningless but series determinations on a single subject reflect significant changes (125, 148). The responsible urine component is ether-soluble and nondialyzable (148). A single case is reported in which excessive fatigue (inability to brush teeth without stopping for rest) was the only symptom of cardiac insufficiency for 23½ months (130).

LASTING CHANGES—THE TRAINED STATE

In respiratory mechanism.—Under continued exertion the acute emphysema in rats is followed by thickened alveolar septa, an increase in elastic fibers, and finally new alveoli. This so-called "sport lung" represents a true hyperplasia (127, 170). Strauss found also marked hypertrophy in the diaphragm of rabbits and dogs. An increasingly greater absorption of oxygen per liter of ventilation marks the progress and state of training (33, 112, 158).

In the circulation.—There was little difference between the circulatory measurements of fourteen college athletes and eleven controls when made under basal metabolic conditions (169). The heart shadows of foremost athletes were found to be longer than the norms; but there was little difference in the transverse measures (44, 160). Enlargements were most pronounced in older athletes and in cyclists, professional boxers, and steeple chasers. Endurance sports enlarge the right heart whereas short intense exertion produce greater change on the left side (146). The hearts of thirty-five champion ice hockey players observed through two months of training became progressively larger and exhibited a definite tendency to change toward the long type (128). Doubling

the blood volume of rabbits and rats by intravenous injections of 12 per cent acacia produced no increase in heart weight. This is believed to discount the importance of dilatation as a stimulus for hypertrophy (54). The ratio of dry heart weight to body weight increased with activity in rats (101). The electrocardiograms of highly trained champions frequently show changes commonly considered pathological, but other interpretations must be found because of the excellent performance of these hearts (55, 160). Increased stroke volume was again shown to be the most important single mark of the trained state (158, 169); whereas an increased diastolic blood pressure after exertion was the outstanding criterion in another study (47).

In the blood.—Though there is no increase in resting alkali reserve in the trained state (133), the degree of reduction in alkali reserve after a standard exercise inversely reflects athletic condition (134). The same is true of other changes which mark the degree of breakdown of homeostasis under stress (29). The highly trained athlete is able to maintain an unchanged internal environment in spite of very strenuous work (93). Evidence is produced (112) that work and life at high altitudes favors the attainment of the trained state. The osmotic resistance of dogs' red corpuscles was unchanged after five minutes and increased after thirty minutes of running; extensive training lowered the resistance, but made it refractory to further thirty-minute running periods (147). Training increases the glutathione content of rat liver, kidney, and muscle, but especially that of blood and heart, 39 per cent and 31 per cent respectively, (126). Sharp immediate reductions in blood glutathione after strenuous exercise distinguish the untrained from the highly trained; in the latter slight rises are followed by only minor reductions (153). Glutathione is liberated from stimulated dog muscles (154).

In muscles.—The altered form of dog muscles (studied in one runner and three controls) as well as skeletal changes are held to indicate that running may actually produce a "runner-type" if begun at an early age (90). The hemoglobin content of rat muscles was increased by a period of activity (101). The conclusion that the phospholipid and cholesterol content of rat muscles is increased progressively over several generations by training is based on a comparison of second and third generation offspring of first generation females "who had run well" with first generation controls and

first generation sacrificed trained rats (17). This unexpected finding deserves the attention of further experimentation in which the influence of "artificial selection" is most rigidly controlled. Training increases the performance of muscles in ischemic states, but far more under conditions of free circulation (103, 118). Muscles which normally are most active in the rabbit such as the heart, masseter, cranial, tongue, and eye muscles consumed up to ten times as much oxygen as did the sartorius and back muscles in Warburg determinations (5).

In metabolism.—The absolute basal metabolism of trained rats was little different from that of unexercised controls; but since their food was limited to the quantities consumed by the controls, their resulting lower weight was responsible for higher oxygen consumption rates when these were calculated to unit weight or surface (16).

In the central nervous system.—Practice was shown (a) to improve mechanical efficiency on a bicycle ergometer by improving skill (178); (b) to guide soldiers in selecting the metabolically most economic step length for every change in road incline (141); (c) to condition athletes to inhale at the physiologically and mechanically most opportune moment in a rowing stroke (85); and (d) to improve the stride and arm-leg co-ordinations in runners (62).

The vascularity of the motor cortex of growing guinea pigs increases progressively in step with the development of motor activity (137). A period of training begun early in life is paralleled by a marked increase of vascularity in the motor cortex and the anterior horn at the level of the fifth cervical segment. This increase is not shared by the sensory cortex (138). This finding parallels earlier observations of increases in heart and skeletal capillarization due to training. The same worker (139) found a rise in liver glycogen content that coincided with the hour usually devoted to exercise. Trained pigs killed at other hours contained no more liver glycogen than their controls.

TESTS OF FITNESS AND THE PREDICTION OF PERFORMANCE

Cardiovascular tests are excellently reviewed and, by the application of Thurstone's factor technique to a large body of data, nine independent factors that are significant in the maintenance of cardiovascular function are analysed; a number of these factors are identified with physiological mechanisms (122).

Pulse recovery after a workout is favored as the best test for athletic efficiency (1). It is interesting that another Olympic champion has likewise found that a certain pattern of pulse rate before exertion and during recovery is most characteristic of athletic prowess (24). The behavior of the electrocardiogram, the x-ray shadow, and the pulse rate during a period of raised intrathoracic pressure is a favored index for predicting athletic ability (2, 3).

The swimming times for distances up to 1320 feet were predicted from (a) maximum oxygen intake while swimming, (b) maximum oxygen debt, and (c) oxygen requirements for swimming various speeds with breath held; the subject never swam more than 180 feet in tests (80).

ENDOCRINES AND PHYSICAL PERFORMANCE

Though for resting or active man the presence of any epinephrine in the blood remains undemonstrated, the closely similar effects of exercise and epinephrine injections on cardiovascular adjustments are taken as evidence for an increased output of this substance during work (26, 27). After enucleation of their adrenal glands, rats performed as well in strenuous work as did sham-operated controls; but in more moderate work they showed an increase in endurance which though "just about significant" statistically is "not understandable" (49). The increased R. Q. subsequent to epinephrine injection during exercise is shown to reflect not merely the driving off of blood carbon dioxide by the lactic acid which follows epinephrine injection but also some true extra oxidation, probably of a part of the lactic acid associated with resynthesis of the remainder (12).

Guinea pigs that had been exercised daily for thirty minutes from the second or third week of life were found at three months to have more than twice as much total capillary length in the zona fasciculata of the adrenal cortex than did controls (14). Bengtsson *et al.* also found evidence that the adrenal enlargement in exercise is a true hyperplasia. The initial increase of blood cholesterol following exercise is regarded as the stimulus causing adrenal enlargement. A much enlarged adrenal subsequently suppresses the blood cholesterol level and further enlargement does not occur (50). Contrary to his own earlier reports Ingle (67) now finds that under more severe tests the removal of one adrenal gland from rats is enough to reduce markedly the work output of the leg muscles.

Administration of a number of gland fractions (66, 68) and several synthetic steroids (65) to adrenalectomized rats proved that certain life and weight maintaining factors could not maintain work output and conversely, some work output-maintaining factors could not maintain life and body weight. No substance or combination of substances restored full work capacity. A high sodium, low potassium diet prolonged the work output of adrenalectomized rats, but did not restore it to normal capacity (69). That the myasthenia of adrenal insufficiency is not due primarily to an increase in muscle potassium is further sustained by the finding that no direct relation exists between the amount of potassium in rat muscle and its response to tetanic stimulation (110). In one man the subcutaneous implantation of pellets of desoxycorticosterone acetate was followed by a remarkable recovery from myasthenia gravis (114). The alkali reserve of normal human subjects was reduced less by a standard exercise when a cortin preparation was administered (132). A period of exercise (1500 m. in 4 hr.) reduced the ascorbic acid content of rat and guinea pig adrenal cortex about 40 per cent. Recovery was incomplete in twenty-four hours, but after forty-eight hours there was a 25 per cent excess. Shorter exercise periods produced milder reductions and more rapid recovery (144).

Whereas strenuous work reduces the phosphorus and glycogen content of rats' hearts significantly, exercise after thyroxin aggravates the reduction of these two substances as well as that of adenylypyrophosphate which without this sensitizing effect of thyroxin is unchanged by work. Thyroxin without work reduces only the phosphorus content (162).

Castrated male animals showed a reduction of about 40 per cent in phosphagen and glycogen content of heart muscle. Injection of "testoviron" returned phosphagen levels to normal and increased the glycogen content far above normal. In noncastrates similar injections doubled heart glycogen content thus involving this hormone as one factor in heart performance (161). Testosterone propionate (Perandren) markedly increased the strength and total work output of two male patients suffering myotonia atrophica and testicular atrophy. Another patient with progressive muscular dystrophy and normal gonads showed no improvement (56). The work output of rat muscles measured by Ingle's method was unchanged after castration of males and females (64).

FOODS, TONICS, AND DOPES IN PERFORMANCE

Performance ability is extended objectively when any necessary mechanism or energy supply is improved. An increased performance not based on some such improvement is due merely to a removal of subjective factors which normally inhibit performance safely within the danger limits. Measures employed to attain mastery by the first of these routes are sanctioned in legitimate training, whereas medicaments used to induce the latter reaction are called "dopes" and are rated as unfair in competition and possibly dangerous to the subject.

From the observance of changes in the performance records of highly trained athletes on various proportions of the food principles, a diet of 150 gm. fat, 700 gm. carbohydrate, and 100 gm. protein plus adequate minerals and vitamins daily, is recommended for distance events, and 100 to 130 gm. fat, 350 to 400 gm. carbohydrate and 210 gm. protein, mostly meat, is recommended for speed events (32). Additional studies of this nature conducted under more rigidly controlled conditions are needed in this very complex field. In nitrogen-balance studies on dogs, high nitrogen diets were found purposeless in work periods and low nitrogen diets particularly undesirable in post-exercise periods. A diet which is just a little above that required for nitrogen balance was found best for maintaining nitrogen balance (122). Not a single vegetarian was found among 158 leading Swiss athletes (177). The ability to perform maximal anaerobic work improved with training over a long period but was uninfluenced by extra gelatin (52). The women in this study did work far in excess of attainments which in men were ascribed by earlier workers to gelatin.

Extra calcium iron and vitamins A, B, C, D, added to the regular army diet of Swiss soldiers produced some measurable improvement in adjustments to work (28). The development of fulminating beri-beri from subacute forms may be hastened by excessive exercise and is paralleled by a marked increase in blood pyruvic acid (98). The perfusion of frog muscles with serum salt solutions containing from .02 mg. to 1.0 mg. per cent of synthetic ascorbic acid caused some vasodilation but no improvement in performance (4). Frog sartorii in Ringer's solution containing .05 to 0.1 mg. per cent ascorbic acid fatigued more slowly than did contralateral controls in plain Ringer's solution. Ascorbic acid

concentrations of 0.5 mg. per cent however, produced an opposite effect (165). Following exhausting ski events, urine ascorbic acid was far below normal for several days (73). Daily extra doses of ascorbic acid given to 572 Bantu mine workers whose normal daily intake of ascorbic acid was below standard did not improve performance in athletic events involving strength, skill, or endurance (76).

By mobilizing phosphorus exercise may support the cure of rickets in rabbits fed a calcium-rich, phosphorus-poor diet (78). Twelve Swiss athletes given calcium gluconate daily throughout a training season showed greater improvement in the ability of the circulatory system to adjust after a run than did fifteen athletes held as controls (106). Contrary to the observations of others, ultraviolet radiation produced no significant change in the resting alkali reserve of eight subjects, but it did improve the body's responses to stress so that a standard exercise caused less lowering of the alkali reserve (136). Some doubt is cast upon the efficacy of peroral sodium bicarbonate to improve performance as claimed by others (135). Digitalis was found to increase alkali reserve slightly. Subjects so treated also showed markedly less reduction of alkali reserve after a standard exercise than did controls (131). Physostigmine increased the performance of rat and frog muscles under artificial stimulation and of athletes' running (10 per cent); in each instance less lactic acid was formed (109).

Pervitin (1-phenyl-2-methyl-aminopropane), an ephedrine derivative, was shown to be a dope; increased performance was always followed by greater exhaustion (21, 96, 171). A woman whose output was trebled apparently was unable without pervitin to force herself into "second wind" (96). It stimulates mental activity, puts off desire for sleep, and makes the individual more reckless in expending his energy.

RELATIONS TO PATHOLOGY

Headache, itch, and urticaria after a few knee bends (99) and urticaria and asthmatic attacks following strenuous physical exertion (30) are cases of allergy to muscular work.

Extensive search of the literature and police autopsy records support the conclusion that not a single death has ever been legitimately attributed to exercise in an otherwise thoroughly healthy person. The increased arterial pressure, the expiratory (Valsalva)

effect, and the gastrocoronary reflex (coronary constriction during marked distension of proximal stomach) are factors which may aggravate already present cardiovascular conditions during strenuous exercise, especially after a heavy meal (75).

Boxing is condemned without reservation because of subacute traumatizations of brain vessels which accumulate to produce a "punch drunk" condition. Such injury is almost inescapable whenever the head is punished. More serious single injuries are also common (74, 82). Remarkable marathon performance is not inconsistent with marked aortic insufficiency with or without an accompanying mitral stenosis (both uncomplicated) (77).

THERAPEUTIC EFFECTS OF MUSCULAR ACTIVITY

Recreational activity has enjoyed clinically observed success in the rehabilitation of psychopathic patients (22). Training in the relaxing of muscles is more effective in reducing neuromuscular tension than is mere rest (71). Considerable reductions in systolic and diastolic blood pressures and in the electrocardiogram voltages have been observed during muscular relaxation (72). Corrective exercise programs extending over periods of months and years were successful in improving markedly the posture of forty-seven out of forty-eight children (23).

BIOMECHANICS OF EXERCISE AND SPORTS

Painstaking mechanical analyses of a single running step indicates the development of 2.61 h.p. of which 0.15 h.p. is employed to overcome wind resistance. The remainder is needed to accelerate and change the direction of various limb portions. This leaves much less if any for the formerly important "viscosity factor" (35). Arm action in running serves to decrease body rotations and in other ways justifies itself as an integral part of the dynamics of progression (36). Electrical and mechanical records of muscle action during running support the view that powerful isometric contractions initiate ballistic movements of the limbs which then carry through under momentum with the driving agonists relaxed (62).

"Big Ten" basketball players travel from 3.46 to 3.89 miles in the course of a game (107). College girls travel from 0.96 to 1.22 miles in a standard game (111). The strenuousness of the two- and 3-court game in girls' basketball was compared. The strenuousness

is related more closely to skill (positive correlation) than to the type of field played. Unexpectedly, the three-court game was slightly more strenuous (59, 111). The force of arm action in various swimming strokes was measured and the mechanical efficiency of swimming was calculated to lie between 0.22 and 1.35 per cent (81). The mechanics of several fancy dives were analysed in terms of the laws governing projectiles and angular velocities (95).

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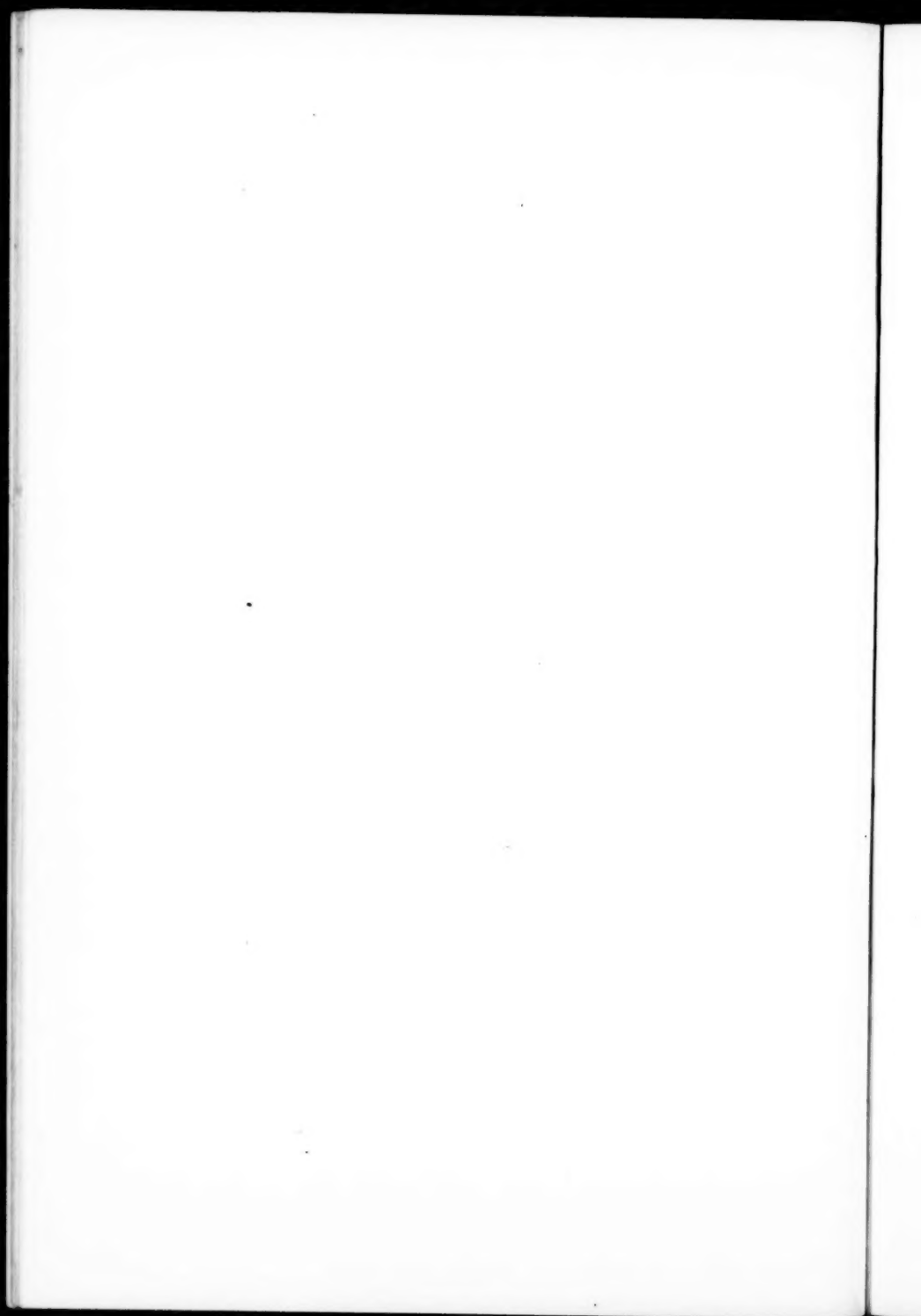
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